

PREFACE

This book treats three different aspects of quality assurance (QA) for environmental analysis: establishment of the state of the art of environmental analysis for a wide variety of elements/compounds, with particular emphasis on chemical speciation, critical evaluation of the current analytical techniques and method performance, and discussions of existing means of quality assurance.

Quality assurance for environmental analysis is a growing feature of the 90's as is illustrated by the number of QA guidelines and systems which are being implemented nowadays. However, there is often a huge gap between the implementation and respect of QA guidelines and the technical approach undertaken to improve and validate new analytical methods. This is particularly true for complex determinations involving multi-step methodologies such as the ones used in speciation and organic analyses.

Quality assurance may also be considered from the technical point of view. The focus of this book is on this approach: the techniques used in different analytical fields (inorganic, speciation and organic analysis) are critically reviewed (i.e. discussion of advantages and limitations) and existing tools for evaluating their performance are described (e.g. interlaboratory studies, use of certified reference materials). Particular reference is made to the Measurements and Testing Programme (BCR) of the European Commission and its efforts to improve the quality control of environmental analysis. Obviously, the number and variety of techniques currently used in environmental analysis is so wide that this book could not ensure a complete coverage.

The book is organized in twenty-four chapters covering various aspects of inorganic chemistry, speciation analysis and organic chemistry as applied to environmental analysis.

The first chapter gives an overview of quality assurance principles as applied to environmental analysis. In particular, the assessment of method performance in interlaboratory studies as well as the use of certified reference materials (CRMs) are discussed. The chapter also describes potential sources of errors occurring in inorganic, speciation and organic analyses.

The following four chapters are critical reviews of techniques currently used in environmental analysis and discuss their performance along with various QA aspects. Recent developments in ICPMS and isotope dilution ICPMS with application to the determination of Pb and Hg in environmental matrices are described, systematic studies for the detection of sources of error in chromium determination by FAAS and ETAAS are reported, and extensive reviews on the analysis of environmental and biological matrices by atomic spectroscopic methods and neutron activation are presented, with particular emphasis on their validation. All these aspects are linked to projects which were carried out within the BCR, either in the form of bursary (Chapter 2) or research contract (Chapter 3), or from the view point of participants in BCR-certification campaigns (Chapters 4 and 5).

Two chapters provide critical reviews on the application of sensors for environmental analysis and field measurements. Although these techniques have not been evaluated so far in classical interlaboratory studies organized by the BCR, the development of new instrumentation is one of the features which is being considered by the Measurements and Testing Programme and hence their inclusion in this book.

The main core of the book deals with QA aspects of speciation analysis. Here again, most of the contributions are closely related to projects currently carried out within the Measurements and Testing Programme (BCR). The different chapters give a critical review of existing techniques and the means of validation that have been developed, either through BCR-interlaboratory exercises or certification campaigns. Four chapters deal particularly with the determination of different oxidation states of elements (antimony, chromium and selenium) and forms of aluminium, of which feasibility studies were successfully carried out prior to the organization of wider interlaboratory studies. Two other chapters give examples of the improvement achieved so far in the framework of BCR interlaboratory studies, namely on arsenic and mercury speciation; these contributions are followed by extensive reviews on techniques used in lead and tin speciation analysis, along with the interlaboratory studies concluded so far. The chapters 16 to 19 deal more particularly with research aspects, either for the development of new techniques such as HPLC-ID-ICPMS for lead and tin speciation and supercritical fluid extraction procedures applied to tin speciation, or the validation of GC-MS for the determination of organotin compounds; a critical review on hydride generation applied to speciation analysis using GC-AAS is also provided. These projects were developed either in the framework of bursaries (Chapter 16) or partially under research contract (Chapters 18 and 19), or following participation in certification campaigns (Chapter 17). Chapter 20 represents a bridge between the inorganic and speciation aspects of environmental analysis; it describes the development of a programme carried out within the BCR which aimed at the harmonization and validation of single and sequential extraction procedures used for the so-called speciation of trace metals in soils and sediments (determination of extractable trace metals) for the assessment of their mobility and bioavailability.

The last part of the book focuses on organic analysis and describes research undertaken to improve the determination of chlorinated biphenyls in air and carbamate pesticides in environmental matrices (both projects carried out in the framework of bursaries), and the development and validation of methods used within the framework of BCR certification campaigns, e.g. PAHs in environmental matrices and dioxins in fly ash.

This book has been written by experienced practitioners. By its nature, it aims to serve as a practical reference for postgraduate students and environmental chemists who need a wide overview of the techniques used in different fields of environmental analysis and existing ways of evaluating the performance of methods for environmental analysis. The critical discussions of the methods described, as well as the development of quality assurance aspects based on the experience gained at the BCR, makes it unique in this aspect.

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Quality assurance for environmental analysis

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Environmental studies cover a broad range of disciplines, e.g. analytical chemistry, geology and biology, and include several aims such as monitoring (routine analyses), research (studies of environmental pathways), modelling etc. Chemical analyses are in many cases the basis of these studies and hence have an enormous economic impact. Consequently, awareness of the need for the quality control of environmental analyses has increased considerably as is illustrated by the multiplication of quality assurance (QA) guidelines, standards and accreditation systems. However, the accuracy of analytical results is, in many cases, far from being achieved. Typical examples have illustrated the lack of accuracy that occurs in the determination of inorganic [1] and organic traces [2] in environmental matrices. These examples are by no means exceptional; the problem is common to many fields of analysis. When results differ so much, they are not trustworthy and poor performance by analytical laboratories creates economic losses: extra analyses, destruction of food and goods, court actions, etc. Moreover, in the past too many wrong but highly reproducible results have led to misinterpretation of environmental processes.

Intercomparisons are a valuable tool to evaluate the performance of analytical methods or the way they are applied in a laboratory as well as for the verification of results. The results of recent intercomparisons have shown that environmental analyses are often far from being accurate. In order to improve the situation a good quality control system has to be introduced in each laboratory. This chapter addresses some of the main aspects of quality control and gives examples of sources of discrepancies in the determination of major and trace elements, chemical species and trace organic compounds. QA aspects of sample collection and storage are obviously another important feature in environmental analyses but they will not be discussed in this chapter which rather focuses on internal laboratory analytical quality control.

1.1 Overview of quality assurance principles

1.1.1 General

Two basic parameters should be considered when discussing analytical results: accuracy (closeness of the agreement between the result of a measurement and a true value of the measurable quantity of an analyte [3]) and uncertainty (expressed as the coefficient of variation or the confidence interval) due to random errors and random variations in the procedure. In this context, accuracy is of primary importance. However, if the uncertainty in a result is too high, it cannot be used for any conclusion concerning *e.g.* the quality of the environment, nor can it be used for the study of environmental pathways. An unacceptably high uncertainty renders the result useless.

A prerequisite for a good result is a correct calibration. Although calibration is considered as being obvious, experience has shown that, with the introduction of modern automated equipment, it has become a part of the analytical chain to which insufficient attention is being paid. In general and over many years the BCR experience is that in 25-30 % of all cases erroneous results were attributed to calibration errors [4]. This observation is particularly meaningful for speciation analysis in which the required pure compounds are often not available. It is perhaps necessary to stress once again that compounds of well known stoichiometry should be used, of which a possible water content is known. Recommendations on available calibrants *e.g.* for inorganic or speciation analyses may be found in the literature [5,6]. When making a stock calibrant solution preferably two stocks should be made independently, one serving to verify the other; when preparing the dilution (preferably gravimetrically) it is recommended that two independent solutions be made, again for verification purposes. The alternative would be to verify the new calibrant solution using the previous one. Calibrant solutions should be made prior to use, even if the solutions are acidified. The laboratory should carefully consider the calibration mode chosen: (i) standard additions, (ii) calibration curve and (iii) bracketing standards. There is no calibration mode that can be recommended in all cases. All suffer from typical sources of error linked to, *e.g.* the linearity of the calibration curve, the chemical form of calibrant added *etc.* Careful consideration should also be given to the choice of the method of calibration: using pure calibrant solutions, matrix-matched solutions or standard additions [2]. Moreover, internal standards should be used when appropriate.

1.1.2 Statistical control

When a laboratory works at a constant level of high quality, fluctuations in the results become random and can be predicted statistically [7]. This implies in the first place that limits of determination and detection should be constant and well known; rules for rounding off final results should be based on the performance of the method in the laboratory. Furthermore, in the absence of systematic fluctuations, normal statistics (*e.g.* regression analysis, t- and F-tests, analysis of variance *etc.*) can be applied to study the results wherever necessary [8]. Whenever a laboratory is in statistical control, the results are not necessarily accurate but they are reproducible. The ways to verify accuracy will be described in the next paragraphs.

Control charts should be introduced as soon as the method is under control in the laboratory, using reference materials of good quality (*i.e.* stable, homogeneous and relevant with respect to matrix and interferences). A control chart provides a graphical way of interpreting the method's output in time (*e.g.* Shewhart-chart), or of detecting

possible drifts in methods (*e.g.* cusum charts), so that the reproducibility of the results and the method's precision over a period of time and over different technicians can be evaluated [8].

A CRM can be used to assess accuracy. It must be emphasized that reproducible or accurate results, obtained respectively with Reference Materials (RMs) or CRMs, are not always sufficient in QA. The composition of the control materials must be close to the composition of the unknown sample. This closeness should involve matrix composition, possibly interfering major and minor substances *etc.*

1.1.3 Comparison with results of other methods

The use of a Shewhart-chart enables the detection of whether or not a method is still in control; it is not, however, able to detect a systematic error which is present from the moment of introduction of the method in a laboratory. Results should be verified by other (independent) methods. All methods have their own particular source of error. For instance in organometallic chemistry, errors may occur for some techniques due to *e.g.* an incomplete derivatization, a step which is not necessary for other techniques such as High Performance Liquid Chromatography (HPLC); the latter technique, however, may have errors such as incomplete separation, which if encountered in the former technique, is to a lesser extent. In the case of inorganic analysis, a possible source of error for *e.g.* spectrometric or voltammetric techniques can be the digestion of the sample, which is not necessary for instrumental neutron activation; this latter technique, however, may have errors which are not encountered in chemical analysis such as shielding and insufficient separation of gamma-peaks.

If the results of independent methods are in good agreement, they are unlikely to be affected by a contribution of a systematic nature (*e.g.* insufficient extraction). This conclusion has more weight when the methods differ widely. If the methods have similarities, such as an extraction step, a comparison of the results would most likely lead to conclusions concerning the accuracy of the method of final determination, not the analytical result as a whole [9].

1.1.4 Use of certified reference materials

Results can only be accurate and comparable worldwide if they are traceable. By definition, traceability of a measurement is achieved by an unbroken chain of calibrations connecting the measurement process to the fundamental units. In the vast majority of chemical analyses, the chain is broken because in the treatment the sample is physically destroyed by dissolutions, calcinations *etc.* To approach full traceability it is necessary to demonstrate that no loss or contamination has occurred in the course of the sample treatment. The only way for any laboratory to ensure traceability in a simple manner is to verify the analytical procedure by means of a so-called matrix RM certified in a reliable manner. The laboratory which measures such an RM by its own procedure and finds a value in disagreement with the certified value is thus warned that its measurement includes an error, of which the source must be identified. Thus, CRMs having well known properties should be used to (i) verify the accuracy of results obtained in a laboratory, (ii) monitor the performance of the method (*e.g.* cusum charts), (iii) calibrate equipment which requires a calibrant similar to the matrix (*e.g.* X-ray fluorescence spectrometry), (iv) demonstrate equivalence between methods and (v) detect errors in the application of standardized methods (*e.g.* International Standardization Organisation, ISO or European normalization Committee, CEN *etc.*).

The conclusion on the accuracy obtained on the unknown sample is always a conservative one: if the laboratory finds wrong results on a CRM it is by no means certain of a good performance on the unknown. If, however, the laboratory finds a value in agreement with the certified value (according to ISO-Guide 33 [10]), owing to discrepancies in composition between CRM and unknown, there is still a risk that the result on the unknown may be wrong. The use of as many as possible relevant CRMs is therefore necessary for a good QA.

1.1.5 Interlaboratory studies

Participation in interlaboratory studies is useful to detect systematic errors. In general, besides the sampling error, three sources of error can be detected in all analyses: (i) sample pretreatment (*e.g.* digestion, extraction, separation, clean-up preconcentration *etc.*), (ii) final measurements (*e.g.* calibration errors, spectral interferences, peak overlap, baseline and background corrections *etc.*) and (iii) the laboratory itself (*e.g.* training and educational level of workers, care applied to the work, awareness of pitfalls, management, clean bench facilities *etc.*).

When laboratories participate in an interlaboratory study, different sample pretreatment methods and techniques of separation and final determination are compared and discussed, as well as the performance of these laboratories. If the results of such an interlaboratory study are in good and statistical agreement, the collaboratively obtained value is likely to be the best approximation of the truth.

Before conducting an interlaboratory study the aims should be clearly defined. An intercomparison can be held [11] (i) to detect the pitfalls of a commonly applied method and to ascertain its performance in practice, or to evaluate the performance of a newly developed method, (ii) to measure the quality of a laboratory or a part of a laboratory (*e.g.* audits for accredited laboratories), (iii) to improve the quality of a laboratory in collaborative work with mutual learning processes and (iv) to certify the contents of a reference material. In the ideal situation, where the results of all laboratories are under control and accurate, interlaboratory studies of types (ii) and (iv) will be held only. For the time being, however, types (i) and (iii) play an important role.

1.2 Sources of errors in environmental analyses

Errors in environmental analyses may occur at different steps of the analytical process, from the sample preparation to the final determination. This section will focus on the specific sources of errors which are likely to occur in inorganic, speciation and organic determinations in the laboratory. The aspects related to the sample collection, storage and pre-treatment will not be developed here. Aspects concerning the necessary precautions to avoid errors in weighing (calibrated balance), volumetric manipulation (calibrated glassware, temperature control), dry mass correction and cleaning of glassware will not be detailed either. Calibration is obviously one of the main sources of error in environmental analyses and requires particular attention as mentioned in the previous above.

1.2.1 Potential sources of error in inorganic analyses

Inorganic analyses may suffer from systematic errors at the sample digestion step (*e.g.* incomplete mineralisation) or the final determination (*e.g.* spectral interference in inductively coupled plasma emission spectrometry).

1.2.1.1 Sample digestion

Nearly all methods of final determination, especially routine methods, require the digestion of the sample. This serves several purposes such as (i) converting all the species in which an element *x* is present in such a way that it becomes present in one uniform and defined form like the element (*e.g.* mercury), the hydride (arsenic, selenium), the cation (cadmium, cobalt, copper ...) or the anion (chromium ..), (ii) eliminating interfering substances from the matrix (*e.g.* complexing agents, precipitants) and (iii) obtaining the element *x* in a homogeneous and easily accessible matrix (*e.g.* solution). The various techniques for element determination do not all require the same degree of sample matrix breakdown, *e.g.* voltammetry is more prone to errors caused by (complexing) organic matrix fragments than flame atomic spectrometry. Certain biotic matrices are sufficiently digested with nitric acid in a bomb if atomic spectrometric methods (atomic absorption spectrometry, AAS and inductively coupled plasma emission spectrometry, ICP) are to be used because possible complexes will dissociate at high temperature, whereas for voltammetry a further digestion with perchloric acid is necessary [12]. The choice of a digestion technique should take into account the objective of the final determination; incomplete digestion procedures, which consume less time and labour, are often acceptable for well-described purpose, *e.g.* Kjeldahl nitrogen content (food analysis), aqua regia soluble element contents (soils and sludges), extractable trace element contents following sequential extraction procedures (sediments) *etc.* However, when the total element contents have to be determined, total digestion procedures must be selected, taking into account factors such as the method of final determination, the matrix composition, the element contents, the possible interferences, the risks of losses or contaminations, the practicality and possible safety hazards in the laboratory.

It is obvious that the risks of errors at the digestion step will increase with decreasing trace element contents. The risks of contamination may be limited by purifying the reagents, *e.g.* by sub-boiling point distillation. Acids such as HClO_4 , HClO_3 , HCl , HF , HNO_3 , and to a lesser extent H_2SO_4 , can easily be purified and are therefore frequently and successfully applied. The choice of suitable vessels for the digestion is also important in trace analysis, particularly the wall material must be chosen to avoid cross-contamination and/or possible losses by adsorption which in turn may cause memory effects. A material such as polytetrafluoroethylene (PTFE), although stable and inert, may gradually increase its surface area (cracks). Glass and especially quartz-glass has more favourable properties but cannot be used if a silica containing matrix (*e.g.* soils, rocks, some plants *etc.*) is to be digested using hydrofluoric acid. Glassy carbon, if sufficiently resistant against the reagents used is often a good alternative [12].

A good way to reduce the amount of reagent, and hence the risks of contamination, is to operate under pressure in closed vessels at higher temperature to increase the oxidation potential and thus the reactivity. Moreover, working in a closed system avoids contamination by air, floating particles *etc.* The demands made on the inertness of the vessel increase considerably when performing pressurized digestions, *e.g.* PTFE has a limited lifetime, glass vessels may release trace elements *etc.* Quartz-glass is a suitable material but cannot be applied for matrices when silica is to be dissolved and a strong attack is necessary, *e.g.* with hydrofluoric acid.

Losses may occur not only by adsorption but also by volatilization, which occurs especially in open vessels (*e.g.* dry ashing, fusion). Depending on the matrix, carefully selected temperature programmes can avoid a detectable volatilization. It is highly

recommended to apply proper ashing acids (*e.g.* mixture of magnesium oxide and nitric acid) in dry ashing procedures. Potentially volatile compounds should be attacked by the digestion reagent at high temperature in high pressure closed bomb digestion; this procedure should be regarded as most effective for trace analysis [12].

Finally, the recent development of microwave digestion procedures has proven to be promising: this technique has the benefits of rapid sample preparation and reduced contamination risks. However, incomplete destruction of the organic matrix or incomplete digestion has been suspected for plant materials [13]. A recent evaluation of microwave digestion procedures has shown that a good recovery of trace elements in various environmental CRMs can be obtained [14].

A preconcentration step is necessary when the concentrations of the elements to be determined in the digest are too low to be determined directly. Techniques such as complexation and solvent extraction, ion exchange, complexation with immobilised reagents and others, are usually the methods of choice. The choice of a particular technique also depends on the particular requirements set by the method of final determination.

A more detailed and critical discussion on digestion techniques can be found in the literature [12,15,16]; Chapter 4 presents some additional discussion on digestions for trace element determinations in environmental and food matrices by AAS.

1.2.1.2 Other sample pre-treatment

No technique is sufficiently robust not to be susceptible to human error. Insufficient care always leads to erroneous results. Examples of errors in sample pre-treatment have been shown, *e.g.* in the case of IDMS, spike addition should be made before a cold nitric acid overnight digestion stage to avoid erroneous low results due to an incomplete binding of the isotope Hg spike to the matrix [17]. In the case of seawater analysis, in methods using a complexation step (*e.g.* AAS, ADPCSV) the naturally present ligand may inhibit the complexation reaction or part of it, thus lowering the results [18]. Laboratories performing analyses of open seawater cannot use their method for estuarine water, although the concentrations of trace elements in the latter case are higher; the reason is that estuarine water matrices contain higher contents of organic matter and that, consequently, incomplete extraction may occur which stresses the need for the verification of extraction yields and for special precautions (*e.g.* destruction step prior to extraction, use of an excess of extractant, back-extraction)[19].

1.2.1.3 Final determination

The methods of final determination also need attention. For example, the volatility of some compounds (*e.g.* chlorides, carbonyls) can cause differences in vapourisation behaviour between the calibrant and the analyte solution, which in turn affects the results for ETAAS; the presence of even weak complexing agents may cause errors in voltammetric techniques and so on. It is necessary that the calibrant and analyte solutions are similar to avoid these types of error.

Other errors which may occur in the final determination (like peak overlap, spectral interferences) are usually well documented. When changing to another matrix with another element pattern, these sources of error should be taken into consideration. Some typical examples of errors in the final determination of trace elements in some environmental matrices have been detected in the course of BCR interlaboratory exercises; some of them are reported below.

In the determination of mercury by ICPMS, interferences in ionisation were observed which were suspected to be due the presence of high amounts of methyl-mercury [17].

Deuterium background correction was not recommended for AAS determination of chromium since the intensity of the lamp is already low at the wavelength used (276 nm), as observed in the analysis of a plankton material [20]. Similarly, in our experience, *e.g.* for white clover analysis [21], problems may occur in the determination of cobalt as the adsorption line of cobalt is close to the end of the deuterium continuum.

Natural ligands *e.g.* in seawater or estuarine water interfere in electrochemical analysis. Some complexes will not be determined at the mercury electrode, or organic matter may adsorb to the electrode surface thus hampering electron transfer. In both cases, the results of the total concentration will be too low. To eliminate these interferences, photo-oxidation of the organic matter by ultraviolet light or a digestion using nitric acid is usually applied [18,19]. Depending on the amount and composition of the organic matter the irradiation of the acidified sample should last 2-6 h; additional treatment with hydrogen peroxide is performed to enhance the breakdown of the organic matter.

1.2.2 Potential sources of error in speciation analyses

The term chemical species here refers to a specific form (monoatomic or molecular) or configuration in which an element can occur, or to a distinct group of atoms consistently present in different matrices [22]. The determination of "extractable trace metals" will also be dealt with although strictly speaking the word speciation cannot be applied in this case. These group determinations, *i.e.* using operationally defined procedures (single and sequential extractions), have often been used in the 80's as a practical compromise to identify *e.g.* "labile" (*e.g.* for aluminium) or "bioavailable" forms (*e.g.* EDTA extractable trace metal contents); in many cases this can give sufficient information to arrive at a sound environmental policy. However, in most cases the determinations have to be more selective and speciation analyses will include forms of elements with different oxidation states (*e.g.* arsenic, chromium, selenium) or individual organometallic species (*e.g.* mercury and tin species).

The basic techniques for speciation analysis were developed in the early 1980's. These include sample pre-treatment steps, *i.e.* extraction either with organic solvents (*e.g.* toluene, dichloromethane) or different types of acids (*e.g.* acetic or hydrochloric acid), derivatization procedures (*e.g.* hydride generation, Grignard reaction), separation steps (GC or HPLC) followed by detection of the compound or element by a wide variety of methods, *e.g.* AAS, Mass Spectrometry (MS), Flame Photometric Detection (FPD), Flame Ionization Detection (FID), ICP, Electron Capture Detection (ECD) *etc.* Each step of the analytical procedure includes specific sources of error; some of them are described below. A detailed discussion of the most recent developments achieved in speciation analysis in the frame of the BCR-programme has already been published [23].

1.2.2.1 Extraction

The extraction, as applied to the determination of *e.g.* organometallic species, should be done such that the analyte is separated from the interfering matrix with neither loss or contamination, nor change of the speciation and avoiding interferences.

A wide variety of acid extraction procedures has been used for sediment and biota analyses particularly when AAS is used as the final determination step; these involved acids such as hydrochloric acid, acetic acid, hydrochloric-acetic acid mixture *etc.* for organotin compounds [24,25,26]. NaOH has also been used [27]. Other procedures, *e.g.*

for techniques involving GC or HPLC, are based on extraction with an organic solvent, *e.g.* dichloromethane, chloroform, toluene, hexane *etc.* [28,29,30]. A good assessment of QA implies that the extraction recoveries are verified; this is usually done by spiking a sample of similar composition to the sample analysed with a known content of the analyte of concern. This is left to equilibrate and the analyte is determined after extraction. The major drawback is that the spike is not always bound to the matrix in the same way as the incurred or endogenous compounds. Alternatively, and only if the extraction procedure does not change the matrix composition and appearance, recovery may be carried out on the previously extracted real sample by spiking, equilibration and extraction. However, the recovery assessment can often be overestimated; CRMs may again be a tool to ascertain accuracy. They are, however, only useful when they contain incurred, and not spiked, species of which the binding to the matrix and the speciation have not changed during the manufacture of the CRM.

The extraction recovery may vary from one chemical species of the same metal to another *e.g.* mono-, di- and tributyltin display different behaviours [31]; consequently, the recovery should be assessed independently for each compound as well as for the compounds together. It is also necessary to find a compromise between a good recovery (sufficiently strong attack) and the preservation of the speciation, *e.g.* the use of too concentrated HCl for the extraction of biological material has been shown to alter the methylmercury content as was demonstrated by the use of radioactively labelled methylmercury [32].

The decomposition during extraction is different according to the types of species. It will be less for stable or inert compounds such as *e.g.* TBT, already considerable for stable or non inert species (*e.g.* chromium(VI)) and paramount for non stable or non inert species (*e.g.* aluminium species).

Clean-up of extracts, *e.g.* by using ion-exchange or other chromatographic techniques was proposed for biological materials; however, this may lead to losses as observed for tributyltin [31]. Clean-up could become simplified or even redundant (reducing the risk of decay) if a more specific method of final determination could be found.

Supercritical extraction could become the method of choice. Such methods are currently being developed *e.g.* for butyltins [33] and are promising for extracting the species without alteration; however, their validation is still under way. The use of such a procedure for the determination of tin species is developed in Chapter 18.

Even if the recoveries of spiked compounds as equilibrated in a certain matrix are total, there is no evidence that the incurred compound will be extracted with the same efficiency. Therefore, a systematic collaborative study of various extraction methods as applied in experienced laboratories in order to validate these methods is necessary. It is stressed here that extraction methods used for speciation suffer in principle from the same sources of error and have the same pitfalls as the methods used for the determination of trace of organic compounds (see below).

Another aspect of extraction concerns the determination of extractable trace element contents, although the term "speciation" is not directly applicable to this kind of determination as mentioned above. The necessity to compare data has led the BCR to support projects for the development and evaluation of common single (soil analysis) and sequential (sediment analysis) extraction schemes which are discussed in Chapter 20. The main sources of error detected in the application of these standardized schemes were mainly due to calibration. Harmonisation, *e.g.* of shaking procedure, duration, choice of reagent *etc.* is necessary [34].

1.2.2.2 Derivatization

Derivatization procedures can be employed to separate trace elements from their matrix, *e.g.* by the generation of volatile species which can be easily separated by gas chromatography. Reactions applied nowadays (*e.g.* pentylation, ethylation, butylation) are mostly centered around the addition of simple groups, *e.g.* with Grignard reagents.

When working with derivatization procedures it should be realised that the reactions are far from being well controlled. The analyst does not know whether in the particular case at hand the binding of the analyte with matrix components is sufficiently accessible to the applied reagent, whether the reaction of matrix components (*e.g.* oils) with the reagent does not prevent the latter from reacting with the analyte, and so on. The reaction mechanisms of derivatization are not well understood and this certainly holds for hydride generation procedures. These procedures are rarely investigated for their traceability. A validation or at least a careful study of the procedure being used in the laboratory for the particular matrix with its particular interferences is necessary. Again, spiking experiments could be used here but, as stated before, they give limited information.

Hydride generation procedures are carried out in acid media, generally using sodium borohydride as reductant and a hydride transfer agent to yield metal and metalloid hydrides. The main advantage of this reaction is that a metal-carbon cleavage does not occur and that the original speciation can therefore be traced back. A wide range of chemical species (*e.g.* antimony, arsenic, germanium, selenium and tin species) can be determined with this approach. By properly adjusting the pH in the reaction vessel the range of compounds that yield hydrides can be made selective, *e.g.* in the case of arsenic for the speciation of arsine and arsenite [35]. However, this procedure presents major drawbacks in the analysis of complex matrices containing high contents of organic matter (see Chapter 19). Sodium borohydride is not able to convert arsenobetaine or -choline into arsine or any other volatile compound. Therefore, hydride generation can be used to separate arsenic species *e.g.* in fish analysis, as only the content of toxic (*i.e.* inorganic) arsenic is of importance; this procedure can also be successfully applied to the determination of methylated arsenic species. However, for the determination of arsenobetaine and choline, hydride generation cannot be applied without pre-treatment, *e.g.* by strong UV irradiation [36,37].

Grignard reactions, *e.g.* pentylation, are widely used for the determination of alkyl-Pb and Sn species as the reaction products can be separated relatively easily by GC. Water has to be removed from the extract as it destroys the reagent and the species of interest; this may be achieved *e.g.* by extraction of a diethyldithio-carbamate complex into an organic phase prior to derivatization (*e.g.* in the case of alkyl-Pb species determination [38]). This back-extraction increases the risks of contamination or losses. In addition, the yield of derivatization should be validated, which is presently hampered by the lack of suitable calibrants (*i.e.* derivatized chemical species). This derivatization procedure is discussed in detail in Chapters 14 and 15.

The use of sodium tetraethylborate overcomes the problem of hydrolytic instability of the Grignard reagents, allowing ethylation to be carried out in an aqueous medium (*e.g.* for the determination of alkyllead, tin and mercury species [39,40]). Here again, the lack of suitable calibrants hampers a verification of the yield of derivatization.

In general, the risk of producing a wrong result increases with the number of steps in a determination and with their complexity. Therefore, if derivatization can be avoided it is worthwhile considering such a possibility.

Other important aspects in the derivatization procedures are linked to calibration and the required determination of reaction yields. In such cases the calibrant should be taken through the whole determination procedure. However in order to be better able to investigate sources of errors, it is preferable to calibrate the final determination step with the compound measured in reality (*i.e.* the derivate) and to run a recovery experiment with the analyte. Although being more time and labour consuming, such procedures should be performed at regular intervals to detect possible errors and they therefore ought to be a part of the laboratory's quality control procedures.

1.2.2.3 Separation

Separation is necessary when the determination of different species cannot be performed with sufficient selectivity or sensitivity, *e.g.* a tin-selective detector (AAS) cannot distinguish between the different forms of tin such as tributyltin, triphenyltin, dibutyltin *etc.* The separation of chemical species of elements can only be performed by techniques which do not alter these chemical forms. Usually, this separation is performed after extraction and a suitable clean-up of the extract by three basic methods: gas (packed or capillary) or liquid chromatography (*e.g.* anion exchange, ion pairing reversed phase), capillary zone electrophoresis (CZE) and cold trapping.

Recent developments in chromatography as applied in organic analysis are not sufficiently applied in speciation analysis. Whereas for most environmental applications packed columns are abandoned for the determination of traces of organic compounds because of poor separation and time-consuming procedures, they are still widely used for speciation. For example, most laboratories still apply packed columns for the determination of methylmercury; after a tedious and badly understood "conditioning" of the column with mercury chloride, the methylmercury species are separated from the other mercury compounds. GC-techniques with a better separation resolution and with a better reproducibility of column performances should be introduced for speciation. The use of capillary columns as well as of internal standards, which is general practice when working with organic traces, urgently needs to be generalized in speciation work as demonstrated in the case of the determination of methylmercury [41].

As in organic analysis, precautions should be taken to preserve the compound integrity in the column. For example in GC-separation of tin species a heat-induced decay may occur leading to the deposition of tin oxides in the capillary column which in turn cause peak tailing.

Thermostable and volatile compounds may be separated by Gas-Liquid Chromatography (GLC). Several stationary phases are available. The separation power usually relies on the polarity of the compounds and of the stationary phase. This separation method, however, often requires a derivatization step.

For LC systems there is no need for derivatization prior to separation. Unfortunately, the choice of stationary phases in HPLC is more limited (*e.g.* ion exchangers, ion pairing) than in GC and, consequently, some separation problems may still exist for several species (*e.g.* arseno-betaine/arsenic(III)). However, LC is better suited for the on-line connection to element specific detectors such as ICP-AES or ICP-MS, AAS, or for Neutron Activation Analysis (NAA) or electrochemical detectors.

Cryogenic separation has been used successfully for the determination of *e.g.* alkyltin and selenium compounds [27], lead and some arsenic compounds [42]. The advantages of this technique are that it concentrates the species and sequentially separates them according to their specific volatility. One drawback is that it requires a derivatization

step; only volatile forms of elements (hydrides, ethylated or methylated forms) may be separated; other molecules of low volatility, *e.g.* triphenyltin, arseno-betaine etc., cannot be separated. Both steps are difficult to validate and it is still unclear which physical and chemical parameters hamper, for a given matrix, the formation and the separation of volatile forms. Moreover, the separation based on the evaporation temperatures may not be sufficient to distinguish two compounds of similar volatility [42]. Although the technique is not always applicable, its simplicity and the fact that it can operate on-line with derivatization steps makes it a recommended method for a variety of compounds.

Other powerful techniques that have proven suitable for a wide range of applications in organic analysis (see section on organic analysis) should be considered for speciation. The power of Capillary Zone Electrophoresis (CZE) has been demonstrated for non-volatile, stable polar compounds. Recently, this technique was successfully applied to the determination of arsenic species [44].

The transfer of knowledge on trace organic determinations to the field of speciation analysis is necessary.

1.2.2.4 Detection

The detectors used for speciation analysis are either element specific (*e.g.* AAS) or non specific (*e.g.* FID, FPD, ECD). In general, the determinand should arrive alone into the detector to avoid interferences; multi-component detection (*e.g.* ICP-AES, ICP-MS, NAA) always has larger uncertainties. In speciation analysis, the choice of the detector strongly depends on the chemical forms to be determined and on the mode of separation used.

Electrothermal AAS (ETAAS), although being a sensitive technique, is generally not recommended for speciation analysis, as the method cannot be applied in a continuous (on-line) mode. The necessary manipulations, caused by the off-line character of the method, increase the risks of errors considerably.

Whenever applied, the measurement precautions are the same as for inorganic analysis; the choice of the matrix modifier, the temperature programme *etc.* should follow the same rules as for the determination of the element content.

Flame or quartz furnace AAS is often used as an element-sensitive detector. When various parameters are chosen and set properly, the technique can be performed on-line; provided that a proper separation (*e.g.* GC, cold trapping) is achieved it can be used to determine the various species containing the same element.

ICP-AES or ICP-MS can be used on-line after HPLC separation, using a proper interface.

MS can be specific and even would allow an on-line QA in the isotope dilution mode.

Voltammetry could be used in some cases as a species-sensitive detector, provided that the species to be determined are sufficiently electroactive and that electrode reactions proceed at a sufficiently high rate. This technique has been successfully used *e.g.* for the determination of tributyltin in water and sediment [45].

Classical detectors may be applied after LC or GC separation for the determination of some chemical forms of elements *e.g.* FPD or FID detection for TBT.

1.2.3 Potential sources of error in organic analyses

In principle, there are no special sources of errors related to the determination of organic compounds in environmental samples. The various steps which can be identified for inorganic or speciation analysis are identical in organic analysis: sample treatment, final detection and calibration.

In practice the analyst is confronted with similar difficulties but with much more limited possibilities to solve them. Organic substances are composed of C, H, O, N, S atoms with the possible addition of other elements like halogens. Only the chemical structure is specific and not the elemental composition. Therefore, the determination of organic substances requires a method that does not alter the compounds to be determined, or only in a controlled and traceable manner. The type of detectors available for organic analysis is rather limited and only very few techniques are specific *e.g.* MS. The specificity of the determination relies mainly on the sample pretreatment, and on the separation of the compounds from the matrix and from possible interfering substances.

Environmental samples are solids, liquids or gases. Gaseous substances may be detected directly. For solids and liquids the analyte is often in a physical stage which does not allow its direct detection. A pretreatment has to be performed. The classical analytical sequence for organic trace analysis is: pretreatment, extraction, clean-up, chromatographic separation(s), final detection and calibration.

1.2.3.1 Pretreatment and extraction of solid and liquid matrices

The objective of the sample treatment is to bring the substance to be determined to the detector in its purest state. The first step is to isolate the substance from the solid or liquid matrix. Especially for solid samples this extraction can pose particular difficulties.

Before the extraction *e.g.* solvent, supercritical fluid, vapor or gas extraction, the matrix may be chemically treated to allow the extractant to come into contact with the substance or to already eliminate some coextracting compounds. No general recipe exists, each sample needs a specific approach. For determining chlorinated compounds, *e.g.* dioxins and furans in incineration fly ash, this can consist of an HCl attack [45]. For materials rich in lipids a saponification *e.g.* with ethanolic potassium hydroxide [46,47] is recommended but high temperatures during the saponification can degrade certain substances *e.g.* dioxins and furans [46]. For animal tissues or fluids a treatment with H_2SO_4 for lipid or protein removal [46,49,50] may be appropriate before or after extraction.

As already mentioned for inorganic analysis, errors may occur during the pre-treatment. When traces or ultra traces of substances have to be determined contamination of the sample becomes a major source of error. Such contamination may originate from the laboratory atmosphere. When low and highly contaminated samples are analysed in the same room such risks are high *e.g.* determinations of pg or fg (absolute) levels of dioxins in food materials and μg levels in waste. Therefore, it is recommended to have separate laboratories for both types of samples. The contamination can also originate from tools and instruments with the sample comes into contact. A good quality assurance and quality control system in the laboratory should avoid that risk; *e.g.* it is good laboratory practice to run procedure blanks in parallel to the analysis of the samples.

Losses may also occur. The substance to be determined may not be totally extracted because the pretreatment was inadequate or too smooth, or because the compound has been altered by the attack. These possible effects have to be investigated in the validation of the method. The extraction of the substance itself is also a source of differences between methods and laboratories. Organic trace analysis often consists in a multiresidue determination and therefore compromises are necessary. In the extraction step, this obliges the analyst to use a proper extraction solvent or an adapted mixture of solvents with an appropriate polarity. For supercritical fluid extraction (SFE) the polarity of the fluid is as important as in solvent extraction but the choice of polarity modifiers is still rather limited. This is a handicap for a wider use of SFE. For liquids the analyst can apply solid phase extraction (SPE) which allows the compounds of interest to be trapped on a solid. This solid should have the property of specifically fixing the compounds of interest so that a primary selection is possible. These systems are increasingly used nowadays for the isolation and the concentration of pesticides from water, contaminants from ambient air, some toxins from body fluids *etc.* The sources of errors lie in their ability to trap quantitatively and to allow a total elution of the substance of interest; the validation of the SPE system has to identify the potential sources of losses. Other extraction methods exist (*e.g.* thermodesorption for chlorinated pesticides or PAHs, sublimation) but are applied only in some special cases [48]. The validation of such methods needs to take into account possible degradation of the substance due to temperature or matrix effects.

One particular aspect of the extraction step of organic substances compared to the determination of single elements is that it is rather difficult, if not impossible, to assess whether the entire substance has been extracted. For solids there are limited possibilities to validate the extraction. Various extractions with different solvents or solvent mixtures can be performed or successive extractions can be carried out, verifying if the successive extracts still contain traces of the substance. This procedure is only helpful to verify that the first extraction was not saturated and consequently part of the substance to be extracted remained in the matrix. If part of the substance is bound to the matrix in a different manner (*e.g.* more strongly adsorbed on clay or organic material) this procedure may not give any additional information towards establishing the validity of the extraction procedure. Spiking experiments can be performed with an estimate of the recovery of the spike. This approach is useful if the spike and the incurred compound(s) are in the same physical stage. Spiking experiments can be elaborated in such a manner that the added compounds are left sufficiently in contact with the matrix so that they approach the stage of the incurred compounds. It is difficult to guarantee that the spiked quantity of substance has been fully introduced into the solid material and no losses have occurred *e.g.* by evaporation, degradation, adsorption on vessel walls or tools. These uncertainties increase when larger batches of materials are prepared in large reaction vessels *e.g.* for the organisation of an interlaboratory study. The analyst can follow the extraction by adding an internal standard. The compound chosen should be absent from the sample and should behave in the same manner as the substance(s) to be extracted (solubility in extraction solvent, extraction kinetics). Its addition should be done in the same manner as the spikes described above. More general indications on sources of errors and possible ways to solve them would require detailed examples. Analytical chemists often develop tricks to evaluate the extraction performance from various matrices, especially for food or feed samples. As an example, dairy laboratories estimate the extraction of organochlorine contaminants by following the extraction of lipids from the milk.

1.2.3.2 *Clean-up and concentration*

After extraction the substance to be determined is present in a liquid, supercritical fluid or, more exceptionally, trapped on a solid. Usually, this stage is not adequate for a direct determination. An additional step has to eliminate possible interfering substances or matrix constituents for a better final separation and detection. Cleaning of the extract can be performed by a liquid/liquid extraction system which can separate substances on the basis of *e.g.* polarity or by a liquid solid system which is usually based on adsorption properties. A common source of errors in the clean-up step is the insufficient cleaning which causes changes in the performance of the chromatographic system by other components. The ideal situation would be that the substances to be determined are placed on top of the GC or LC column in a solution identical to the calibration solution so that the system has only to separate the compounds to be determined. This is very difficult, if not impossible, to achieve as the initial sample composition is generally largely unknown. The analyst has to verify that the final detection is not influenced by any remaining matrix constituent or solvent. Classical difficulties are the presence after clean-up of residual alkanes, lipids which influence the detector response, *e.g.* electron donors for ECD leading to negative peaks in the chromatograms, contamination of the ECD, overloading of the ionisation source of the MS *etc.* Usually, insufficient clean-up can be detected by inspecting the chromatograms: irregular and important background, unidentified peaks, low response of the substances to be determined compared to the standards, erratic behaviour of the detector *etc.* To avoid such problems the analyst has to modify the clean-up or investigate if the material used is in the adequate stage (*e.g.* activity of silicagel or alumina) or present in a sufficient amount. An exaggeration of the clean up by applying overly long procedures may lead to losses of substance on the clean up materials. Usually, several clean-up systems can be applied, they have to be adapted to the previous extraction and to the following chromatographic systems. Several interlaboratory studies within the BCR have proven this. These studies have also demonstrated that a minimum of quality control of the chromatograms allows inadequate cleaning to be detected [47,51,52].

Another source of error in the clean-up step is an inappropriate elution. Inadequate solvent or premature cut-off of the elution can lead to losses of substance and consequently to inaccuracy.

In several cases, and in particular for the determination of traces of organic compounds in environmental matrices, it is necessary to concentrate the extract before the clean-up or before the chromatographic separation. This step may be critical as it consists, except in the case of SPE, in the elimination of a part of the solvent. Organic substances, especially those determined after GC separation, are volatile compounds. Therefore, evaporation of the solvent can lead to losses. A general rule is that the concentration by heating should never go until dryness as heating of the residue may lead to evaporation or decomposition of part of the substance (*e.g.* rotary evaporation should not go below 5 ml). The final concentration of the extract should always be performed under a stream of clean nitrogen or argon of verified purity. In case of very volatile substances and unless fat is present in the extract, it is possible to add a clean keeper *e.g.* high boiling point solvent with 12-14 carbons, which will avoid evaporation of the substance. Evaporation devices should always be perfectly clean as they are a frequent source of contamination.

Clean-up and enrichment steps can be validated through relatively simple techniques compared to the extraction. Spiking with labelled compounds, standard additions, and internal standards can help to validate and to control the clean-up step.

In general the interlaboratory certification studies in the field of organic analysis have shown that the clean-up step is not the most important source of errors and disagreement between laboratories. Extraction efficiency and certainly chromatographic separation and calibration are far more important and frequent sources of inaccuracy and of bad precision. The importance of a proper calibration is often ignored which is the reason for errors which may relatively easily be avoided.

1.2.3.3 Separation

Specific detectors for the determination of organic substances are limited to mass spectrometry. To obtain a signal traceable to a pure substance it is necessary to bring the substance free of any interference to the detector. This is the role of the separation step.

Separation systems applied to organic analysis can be grouped into LC (mainly HPLC), GC, supercritical fluid chromatography (SFC) and capillary zone electrophoresis (CZE). Thin layer chromatography (TLC) is rarely used but HPTLC (high performance TLC) and bidimensional TLC systems develop rapidly and are used for PAH determinations [47]. CZE is still an experimental technique for organic trace analysis but is very promising for e.g. peptides and drug residue analysis in animal tissues. Liquid and gas chromatography are the two main separation systems used.

Both techniques have a large spectrum of applications but often in separate fields. Therefore, they are complementary. GC is limited to compounds which are gaseous at temperatures compatible with the GC phases and HPLC is mainly used for thermally fragile compounds. Some families of compounds can be analysed in both systems (e.g. PAH, phenols). In both techniques the main source of errors lies in the inability of the chromatographic phase to separate some compounds. Interferences can be minimized by changing the phase or other column characteristics e.g. column length, diameter, particle size, film thickness *etc.* or the separation program parameters such as temperature, elution gradient *etc.*

In some fields of organic trace analysis the state of the art of GC is the limiting factor. This is the case for dioxin and furan determinations where progress in GC phases allows a step forward in the accuracy of measurements. Figure 1 shows the difference between two types of polar columns; one SP 2331 (Supelco) on chromatogram A and two DB Dioxin (J&W Scientific) columns: one DB Dioxin column (chromatogram C) being brand new and the other one having served for several runs (chromatogram B). Figure 1 also highlights the importance of the column maintenance and the rapid degradation of the separation power. In the case of polychlorodibenzo furan (PCDF) separations this degradation for the older column led to a shoulder of the peak of F118 by F119 where the new column still offers a separate peak for F119.

Similar situations have been encountered in other certification studies where the results of several laboratories could be compared. In the determination of chlorobiphenyls, using polar columns it could be shown that the certification some years before of CB 138 was wrong due to the unexpected presence of CB 163 [52]. Similar difficulties were encountered for the certification of benzo fluoranthene b,k and j isomers with some GC systems [53]. Such difficulties in the separation of isomers or congeners can be solved by the use of multidimensional GC or by applying an HPLC "fractionation" of the

cleaned extract before GC separation [48]. Such methods are time-consuming and can hardly be applied in daily routine determinations. For certification, however, they are a must and through the availability of CRMs, normal routine measurements profit from these achievements.

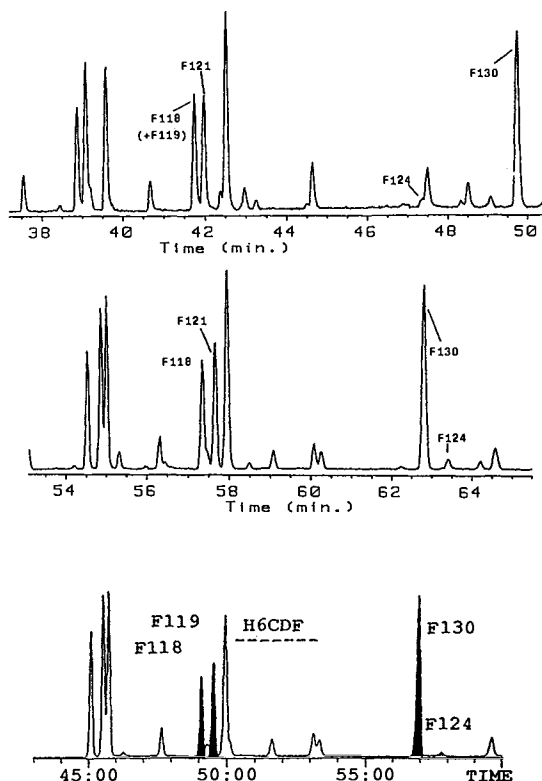


Figure 1: Separation of furan isomers in an incineration fly ash (BCR-CRM 429). Difference between two types of polar columns; one SP 2331 (Supelco) on chromatogram A and two DB Dioxin (J&W Scientific) columns: one DB Dioxin column (chromatogram C) being brand new and the other having served for several runs (chromatogram B). This highlights the importance of column maintenance and the rapid degradation of the separation power.

Additional sources of errors exist in chromatographic systems. The difficulty to achieve reproducible injections in GC drastically affects the precision of the method if no internal standard is used. There are still analysts who claim that internal standards are useless in GC, that they only add additional uncertainties into the determinations due to their own detection and their potential interference with the sample peaks of some samples.

Figure 2 shows an example of results of an interlaboratory study where one laboratory (lab Nr 11) did not use any internal standard. The automatic injection systems used in HPLC are more reproducible so an internal standard for the correction of the injector fluctuations is not necessary, but only on condition that the analyst has verified that it works properly before running the system .

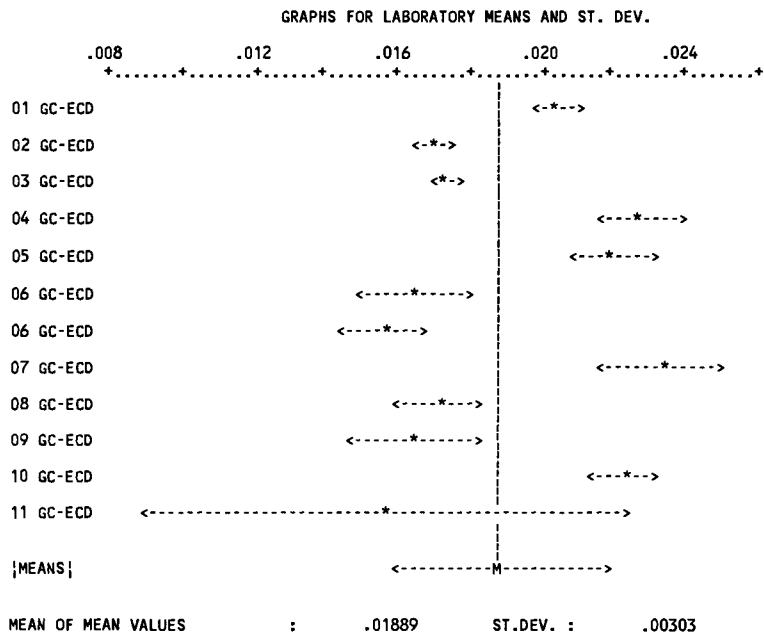


Figure 2: α HCH in animal feed in mg/kg (BCR interlaboratory study, June 1993). Laboratory 11 did not use any internal standard which may explain the large standard deviation compared to other participants.

Other difficulties exist in GC injection. Some compounds are sensitive to elevated temperatures and may be degraded in the injector when split systems are applied. This is the case of p,p'DDT which decomposes partly into p,p'TDE. Often the decomposition of p,p'DDT cannot be quantified as it represents only a small part of the total but it drastically affects results for p,p'TDE which is only present at much lower levels. This decomposition is influenced by the matrix of the sample, changes from one injector to another and depends on the age of the injection liners. As a consequence it is difficult to evaluate the decomposition rate of p,p'DDT in the system and the resulting p,p'TDE measurements are inaccurate. Figure 3 shows results of an interlaboratory study where these decomposition effects could clearly be traced back because the maximum possible concentration of p,p'TDE which resulted from the enrichment procedure was known.

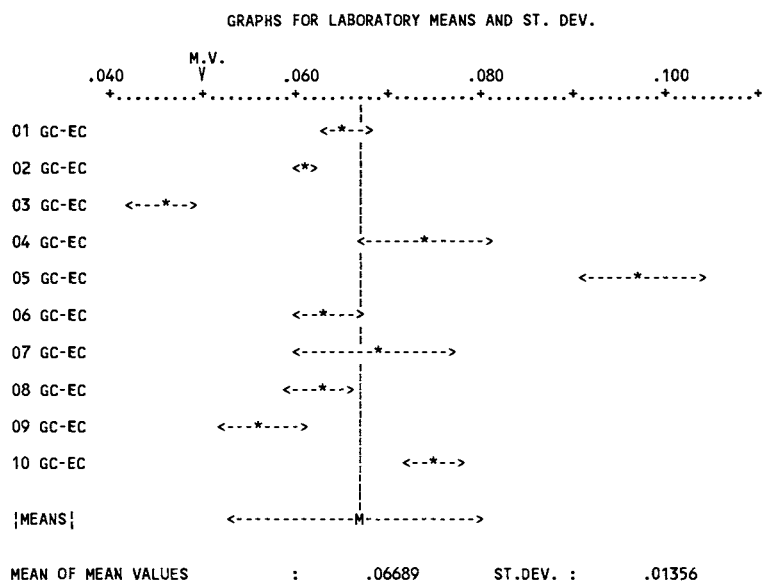


Figure 3: Determination of p,p'TDE in animal feed; effect of p,p'DDT decomposition in the injection systems on the TDE determination. All laboratories except lab 09 (on column) used splittles injection. The maximum possible value (M.V.) for p,p'TDE was 0.05 mg/kg (calculated from the amount spiked into the pesticide free artificial animal feed).

1.2.3.4 Final detection

After separation the final detection allows quantitation. The reliability of this step lies in the absence of interferences or matrix effects and in the proper calibration.

The determination of organic compounds relies on few types of detectors which, with the exception of mass spectrometry (MS), are usually not compound-specific, *e.g.* electron capture detector (ECD), nitrogen phosphorus detector (NPD), flame ionisation detector (FID), thermal conductivity detector (TCD), flame photometric detector (FPD) mainly for GC; and UV, fluorescence or diode array detectors for HPLC.

MS (MSD, High Resolution MS, tandem MS) is mainly used in association with GC. Coupling MS to HPLC or CZE is becoming increasingly popular but the MS/LC interface is still a limiting factor.

Major sources of errors related to the detection lie in insufficient knowledge of the optimal working range of the detector *i.e.* range of linear response or its improper use. Because of the absence of specificity of many detectors the identification of the compounds is based on the retention time of each substance. Therefore, the use of internal standards is of great importance. The relative position of the substance peak towards the peak of the internal standard allows identification of the peak and also evaluation of the performance of the separation system. Insufficient clean up may result in coelution of substances and can mask or contaminate the detector. The result will be a difference between sample and calibration solutions, a high background signal, anarchic

base lines and in some cases appearance of negative peaks which may also affect the accuracy of the determination. Figure 4 shows such effects due to bad clean-up. In MS systems and especially in HRMS a bad clean-up will affect the efficiency and the stability of the ionisation process and contaminate the system.

1.2.3.5 Calibration

As in other fields, errors in calibration may drastically affect determinations of organic traces. Proper calibration is a difficult task, though underestimated, and requires an experienced analyst. Special difficulties in organic trace analysis are mainly related to the handling of volatile substances, their instability, their storage and transfer. In solution some substances are sensitive to UV light (some PAH, dioxins) or even to ambient temperatures. Therefore, they need to be stored in cool, dark places. Without proper handling of stock and working protocols, standard solutions are a common and important source of inaccuracy. Wells *et al.* [54] have investigated these difficulties and have given advice on how to handle properly organic standard solutions. This was a result of an interlaboratory study on calibration of PCBs organised by the BCR. Figures 5a and 5b show the results obtained for CB 28 and CB 138 in this interlaboratory study for an *iso*-octane standard solution of metrologically known composition. This solution was distributed to 24 European laboratories which used their own calibrants to determine CB (IUPAC number) 28, 52, 101, 105, 118, 128, 138, 149, 153, 156, 170 and 180. This exercise revealed that calibration remains one of the essential sources of disagreement between laboratories in CB determinations. This conclusion may be similar for many other fields of analytical chemistry.

It is evident that at the beginning the analyst should use compounds of certified purity and stoichiometry, or when they exist, certified solutions (calibrants from EPA, NIST, BCR *etc.*). When preparing working solutions he should avoid serial dilutions because an error in the first dilution will be carried over to all resulting solutions.

The analyst should carefully select the internal standard used for quantitation and introduce it into the calibration and the sample solution on a mass basis. The signal of the IS should be similar in intensity to those of the substances in the sample. At this step of the procedure many errors occur. This IS will help to correct for injection fluctuations and to follow the validity of the chromatographic separation. Determinations of organic traces (at least by GC) without the use of any internal standard are unreliable.

Another frequent mistake in organic trace analysis is due to the automatic integration and the base line selection. In cases of fluctuating background, insufficiently separated or deformed peaks, the analyst should first investigate whether peak height or area is better suited and he should manually verify the integration. For economic reasons often only one calibration point is used. This may only be acceptable if it has been verified beforehand that the signal lies within the linear response range of the detector. If this is not the case multiple calibration should be performed or bracketing standards (just above and just under the signal of the compound in the sample) should be used. Organic analyses are time consuming because of the chromatographic separation. Therefore, the analyst should regularly recalibrate the system, or after having passed the last sample a calibration solution should be injected to verify that no drift took place (bracketing in time).

Calibration is one of the most important steps of an analysis and is essential in the chain of activities to guarantee that the expression of the result is linked to known units *i.e.* fractions of a mole of the pure substance.

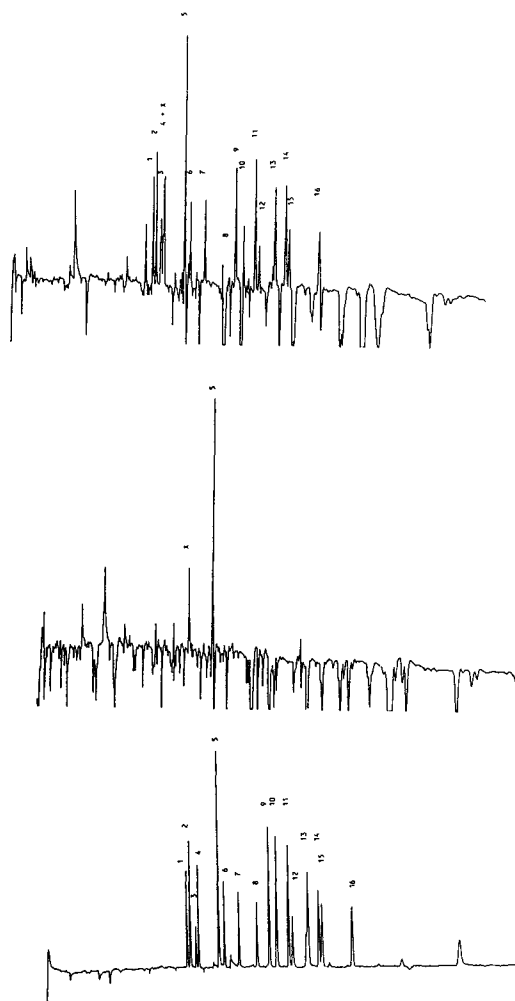


Figure 4: Influence of matrix components due to insufficient clean-up on the ECD response. Upper spectrum: animal feed enriched with chlorinated pesticides; middle spectrum: same animal feed without pesticides; lower spectrum: standard solution. The animal feed contained large amounts of fatty material which was not removed by the applied clean-up procedure. The chromatogram shows numerous negative peaks which enabled a reliable determination.

1: α HCH, 2: HCB, 3: β HCH, 4: γ HCH, 5: internal standard, 6: heptachlor, 7: aldrin, 8: β heptachlorepoxyde, 9: γ chlordane, 10: α endosulfan, 11: p,p'DDE, 12: dieldrin, 13: endrin, 14: p,p' TDE, 15: o,p'DDT, 16: p,p'DDT, X: non identified compound.

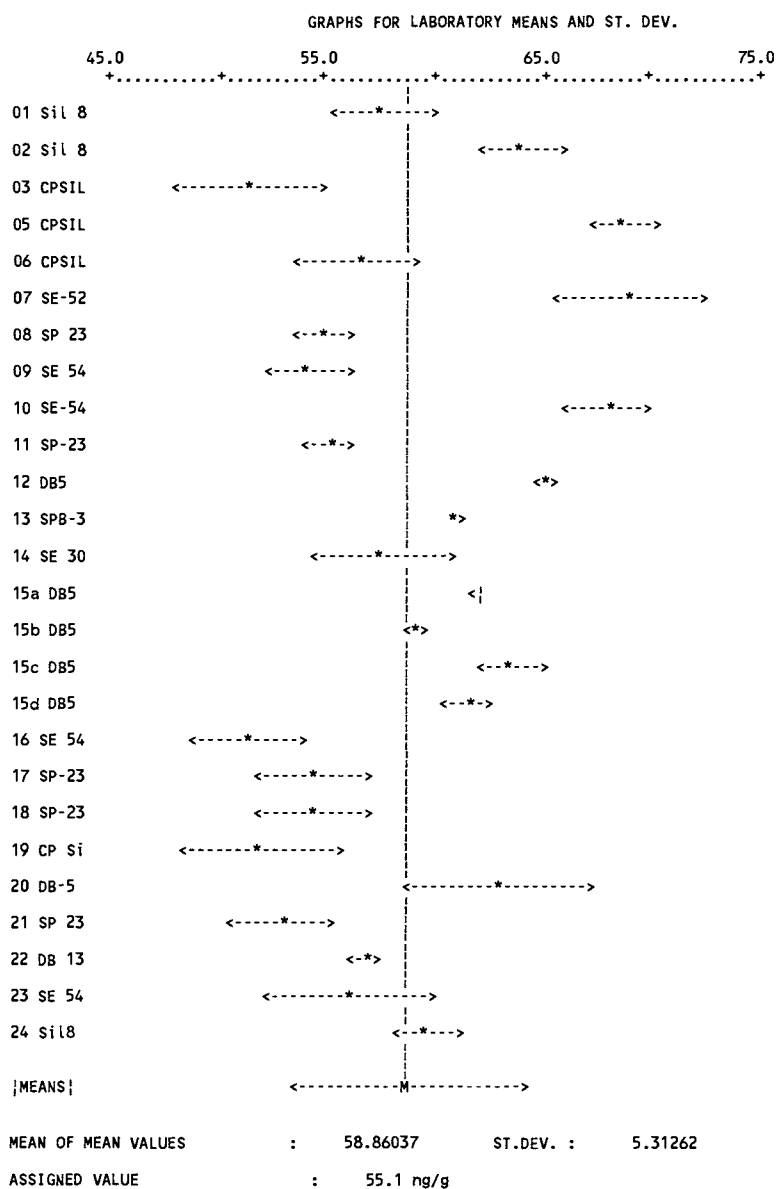


Figure 5a: Graph presentation of the results of the interlaboratory study organised by BCR on calibration of PCBs in *iso*-octane. For CB 28 the between laboratory standard deviation is large (CV of ca. 10 %) and the mean of means different from the assigned value.

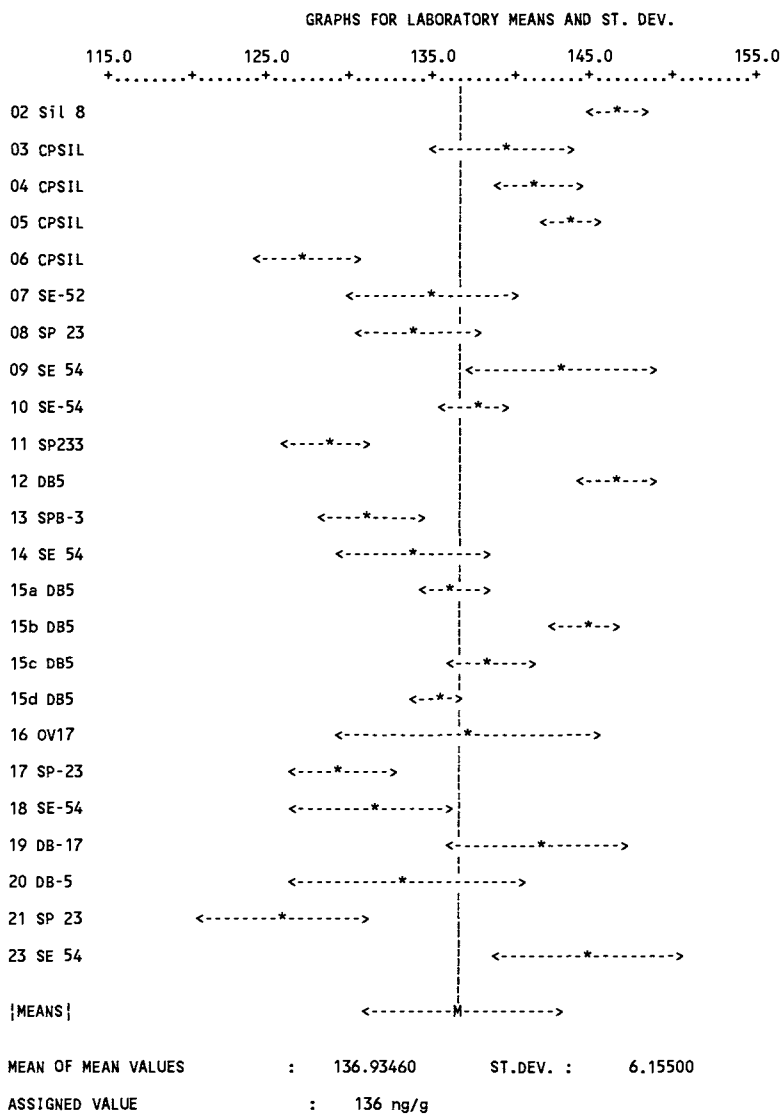


Figure 5b: Graph presentation of the results of the interlaboratory study organised by BCR on calibration of PCBs in *iso*-octane. For CB 138 the between laboratory standard deviation is small (CV of ca. 5 %) and the mean of means agrees fully with the assigned value.

1.3 Conclusions

The need for the harmonisation of measurement systems has led to the establishment of measures to verify a laboratory's performance with regard to quality assurance rules and guidelines (e.g. ISO 9000 and EN 45000 standard series), accreditation systems and the production of certified reference materials (CRMs). The efforts are increasingly focused on an overall approach to quality assurance (e.g. Total Quality Management principles). Besides the aspects of quality related to management, these principles involve an assessment of quality assurance (QA) from (i) sample collection and storage, (ii) analytical work to (iii) treatment of the data. The first item (i) is being considered by normative bodies (e.g. by CEN); however, very little practical work has been done so far and, hence, considerable efforts are deemed necessary. The QA at the level of the laboratory (ii) is more advanced thanks to the different systems already mentioned; it involves internal quality control (QC) principles and external assessment schemes (participation in interlaboratory trials or proficiency testing). This chapter has dealt essentially with the work still to be done at the laboratory level. It illustrates that the quality control of many determinants in environmental matrices is far from being achieved. Quality assurance for environmental analysis is still a challenge that has to be constantly faced. Education, training, an increase in the number of interlaboratory studies and in the production of CRMs are some of the major requirements in analytical chemistry for routine environmental analysis in the next decade.

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2.

Isotope Dilution analysis and ICP-MS: an analyst's dream ?

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The focus of this book is to describe the state of the art for achieving accurate, precise and universally accepted analytical results for a wide range of analytes in all imaginable matrices. Clearly, no panacea technique will emerge which can meet these criteria for all analytes, inorganic, organic and biochemical and so whilst the goals, principles and tools set out in chapter one can be used to form a universal strategy to tackle the problem, methods have to be devised within each specific domain to meet these challenges. In the field of inorganic trace element analysis and in metrology more generally, the best way to minimise analytical errors associated with sample preparation prior to analysis is to use the minimum amount of sample processing possible. Once a solution is prepared for analysis, errors can arise from a number of sources such as: matrix effects causing analyte suppression or enhancement and instrumental drift or instability. Clearly, if any of these problems affect the calibrant and samples to a differing extent then the final result will be incorrect. With all analytical techniques, these problems can be identified by the use of appropriate well characterised reference materials. Where possible, use of a well chosen internal standard element or the standard addition technique will improve the quality of the result when in the hands of a competent analyst. Use of instrumentation that is capable of accurate and precise isotopic ratio analysis offers the analyst a potent weapon in the battle for analytical excellence: isotope dilution analysis. The use of this technique in conjunction with ICP-MS will be discussed in this chapter.

2.1 Principle of isotope dilution

Isotope dilution analysis is a powerful analytical method capable of accurate and precise determinations of analytes that have more than one stable isotope. The principle of the method is that by altering the natural ratio between two isotopes in the sample, by the addition of an accurately known quantity of an isotopic spike, and measuring the ratio of the mixture, the concentration of analyte present in the original sample can be deduced from a knowledge of the weight of sample taken, the quantity of spike added and the ratio of the isotopes in the original sample, spike and mixture:

$$M_s = \frac{{}^bX_t A (R_m - R_t)}{{}^b f (R_s - R_m)} \quad (1.1)$$

Where:

M_s is the mass of analyte present in the sample

bX_t is the number of moles of spike isotope b (tracer) added

A is the relative atomic mass of the element (in the sample)

R_m is the isotopic ratio of the mixture

R_t is the isotopic ratio of the spike (tracer)

R_s is the isotopic ratio of the sample

${}^b f$ is the isotopic abundance of isotope b (used for spiking) in the natural sample.

From the derivation of this equation [1] it can be seen that mass fractionation considerations cancel out in the case of ICP-MS determinations and need not be taken into account. However, for analyte elements that do not have a fixed ratio in nature (*e.g.* Pb) mass fractionation has to be corrected for when determining the relative atomic mass of the analyte (on the basis of the set of observed isotope ratios). It is good practice to analyze an isotopic ratio standard reference material with each set of isotope dilution experiments.

The two major advantages of the isotope dilution over conventional analysis against a set of standards, stem from the fact that ratios, rather than absolute sensitivities, are measured. Firstly, once the spike has been added to the sample and allowed to come to equilibrium, losses of analyte become unimportant since the isotopic ratio of the mixture would not be altered. The second advantage is that the determination is largely unaffected by changes in instrumental sensitivity and matrix effects since both isotopes should be affected to the same extent and so the ratio will remain constant.

2.2 Choice of instrumentation

The use of isotope dilution with thermal ionisation mass spectrometry is capable of providing very accurate and highly precise analyses with typical RSDs of less than 0.1% on the measured isotope ratio. However the technique has some drawbacks. Usually the analyte has to be separated from its matrix and then mounted as uniformly as possible on the filament. Clearly this procedure often involves considerable sample pre-treatment and

chemical separations, is time consuming and increases the risk of contamination (although as stated above, the technique is largely immune to errors due to loss of analyte after spiking, it must be remembered that contamination of the sample with exogenous analyte will result in erroneously high results). The analysis time is relatively long, often taking several hours and consequently sample throughput rates are low, even if multiple filament devices are used.

The quadrupole mass analyzer used in ICP-MS separates ions on the basis of their mass to charge ratio thereby (in principle) providing isotopic composition data for all elements present in the sample. The method of isotope dilution analysis is therefore directly applicable to ICP-MS. The advantages of Isotope Dilution Inductively Coupled Plasma-Source Mass Spectrometry (ID-ICP-MS) over thermal ionisation IDMS are firstly that sample pretreatment, other than dissolution, is not usually required and secondly the sample analysis times are much shorter, of the order of ten minutes for introduction of a sample, five replicate measurements, washout and introduction of the subsequent sample which allows a much higher sample throughput to be achieved than is possible with TI-IDMS.

2.3 ID-ICP-MS

2.3.1 *Precision and performance*

In general, the precision obtained for isotope dilution analysis using TIMS is slightly superior to that for ICP-MS but the precision for a ratio measurement on a single sample mounted on a filament is unsurpassed for TIMS (e.g. $^{87}\text{Sr}:^{86}\text{Sr}$ 0.0039% RSD with TIMS [2] compared to typically 0.1 to 0.5% for ICP-MS). The reason for the superior precision that can be obtained by TIMS lies mainly in the stability of the ion source. Under the right conditions, it is possible to achieve fairly uniform ion production from the filament and the use of time of flight mass spectrometers with multiple charge collection devices means that both isotopes in a ratio can be measured simultaneously, thereby minimising the superimposition of instrumental noise on the system. Inherently, the ICP represents a much more noisy source the performance of which can be altered by many factors (e.g. nebuliser flow rates, alteration of viscosities between samples, matrix effects *etc*). The influence of some of these parameters was studied by Vanhaecke *et al.* [3] in the development of their so called "zone model" which attempts to rationalise these effects in terms of the spatial displacement of a zone of maximum intensity for an ion signal caused by such alterations. Since Vanhaecke suggests that the optima for different elements occur at different spatial displacements and hence ion sampling is a compromise process, matrix effects that cause enhancements for certain elements and suppressions for others in a given matrix can be rationalised.

Begley and Sharp [4] studied the sources of noise in ICP-MS using noise spectral analysis. This information was used to devise a measurement methodology for sequential measurement of isotopes which minimised deleterious effects of non-random instrumental noise on the measured ratio. By careful choice of the acquisition parameters for the peak jumping mode (where the quadrupole is set to do a series of measurements over a peak of

interest and then is "jumped" to the next mass of interest without collecting data from intervening masses, thereby maximising the duty cycle of the mass spectrometer) they were able to achieve a measurement precision of 0.05% for the $^{107}\text{Ag}:^{109}\text{Ag}$ ratio (about one order of magnitude better than normal). They found that ratio precision was limited by inaccuracies associated with the operation of the quadrupole and a component of statistical error arising from the random arrival of ions at the detector.

2.3.2 Routine applications

The capability of ID-ICP-MS will be demonstrated in this section by taking some examples from the literature. In this context, the term "routine" is taken to refer to applications where the researchers encountered no specific or unforeseen problems.

The theory and application of the isotope dilution method to general analytical chemistry was reported by Heumann [5] and includes a discussion of alternative ionisation sources such as spark source, electron impact and field desorption. A review paper by Fassett and Paulsen [6] deals with the accuracy that can be obtained with the isotope dilution technique. As ICP-MS has become more widely established in the analytical community, the number of papers reporting the use of isotope dilution in conjunction with ICP-MS has steadily increased, reflecting the potential of the technique. Van Heuzen *et al.* [7] reviewed the theory of isotope dilution and the precision and accuracy obtainable with the ID-ICP-MS technique, the work was based on an earlier paper by De Bièvre and Debus [8]. Van Heuzen *et al.* used isotope dilution to determine the amounts of Re and Pt on two different catalysts, alumina and silica-alumina and found good agreement with the routine wet chemical analysis and reported a relative standard deviation of less than 0.5% for the isotope dilution analysis.

Okamoto [9] used isotope dilution to certify the tin content of an NIES fish tissue reference material (NIES No. 11). He reported a Sn value of $2.37 \pm 0.04 \mu\text{g.g}^{-1}$ which compared well with the consensus value of $2.4 \pm 0.1 \mu\text{g.g}^{-1}$ obtained using four different techniques (including ID-ICP-MS). Beauchemin *et al.* [10] compared external calibration and isotope dilution techniques for the analysis of twelve elements in two marine biological materials (NOAA L, shellfish tissue and NOAA K, cod liver tissue) and concluded that both methods gave acceptable results, but that the isotope dilution results were more accurate and precise than those obtained by external calibration, particularly at low concentration levels. A similar conclusion was reached by Makishima *et al.* [11] who determined traces of gallium in "pure" aluminium using isotope dilution and standard addition methods. When combined with solvent extraction using isopropyl ether, the method was able to detect $0.029 \mu\text{g.g}^{-1}$ Ga in a 99.999% aluminium matrix. These authors commented that the precision obtained with isotope dilution was superior to that for standard addition, but that both methods gave comparable accuracy, solvent extraction was required in both cases since the gallium signal could not be seen in the aluminium matrix due to signal suppression.

The suitability of ICP-MS for on line isotope dilution using flow injection techniques has been studied by Barnes' group [12,13] at the University of Massachusetts in the USA. In this system, the spike can be added to the sample on line via a second channel of the flow injection manifold and is allowed to mix in a merging stream. The ratio of sample to spike volume can be adjusted volumetrically using microprocessor control via the flow injection

pump [13]. In the preliminary study [11] this ratio was kept to unity (therefore the spike to reference isotope ratio was not controlled). In the paper, Lasztity *et al.* reported that the precision of the method was better than 1% when steady state merging stream conditions were obtained. The accuracy of the technique was verified by analysis of a range of 7 reference materials for which good agreement with the certified values were obtained. One advantage of the flow injection technique is that since sample sizes are limited, the formation of polyatomic interferences (and orifice clogging problems) are kept to a minimum, ensuring better analytical performance. In this type of analysis, it is assumed that the sample and spike do not need to come to equilibrium since aerosol reaching the plasma will be representative of the total and isotopic composition of both components of the sample stream. An example will be discussed later in this chapter which may call this supposition into doubt.

Ward and Bell [2] evaluated ID-ICP-MS as an alternative to TI-IDMS for the analysis of rubidium in geological samples and reported that the precision could be improved by a factor of three from 0.6% with TI-IDMS to 0.17% using ID-ICP-MS. They attribute this improvement to the facility in ICP-MS to compensate for mass fractionation by the analysis of an isotopic reference material whereas in the case of the TI-IDMS analysis such a correction was not possible since rubidium only has two isotopes and the ratio is perturbed by addition of the spike. In TIMS, the extent of mass fractionation is, at least in part, a function of the sample loading onto the filament and therefore cannot be compensated for by the subsequent analysis of an isotopic standard. However, when an unaltered pair of isotopes is present in a sample it can be used as a fractionation monitor if the value of the ratio is fixed in nature and known.

The performance of ID-ICP-MS relative to TI-IDMS is illustrated on table 1 which compares these results against their certified values for a number of BCR certified reference materials (experimental details have been given elsewhere [1,14]). In general it can be seen that the agreement between the ID-ICP-MS and TI-IDMS data is good and both agree well with the certified values. In the case of the estuarine and river sediments, the discrepancies between the results highlight the problems of ensuring total dissolution of analyte in these particular matrices, a pre-requisite for accurate isotope dilution data. Although the ID-ICP-MS value for lead in cod muscle agrees well with the certified value the uncertainty on the result is relatively large (as is the case for the certified value) reflecting that the technique was working towards the limit of its accurate quantitation range.

Table 1: Comparison of results for lead in various BCR reference materials by ID-ICP-MS and TI-IDMS

Sample	ID-ICP-MS Pb $\mu\text{g}\cdot\text{g}^{-1}$	n	TI-IDMS Pb $\mu\text{g}\cdot\text{g}^{-1}$	Certified value Pb $\mu\text{g}\cdot\text{g}^{-1}$
RM 60 Aquatic plant	64.1 ± 1.4	3	64.8 ± 0.4	63.8 ± 3.2
RM 61 Aquatic plant	62.4 ± 0.7	3	61.1 ± 1.5	64.4 ± 3.5
RM 62 Olive leaves	25.7 ± 0.4	3	26.4 ± 0.5	25.0 ± 1.5
RM 277 Estuarine sediment	137.7 ± 1.4	9	148.1 ± 0.9 145.0 ± 3.04 134.7 ± 0.57	146 ± 3.0
RM 280 Lake sediment	76.4 ± 1.1	9	82.7 ± 0.41 81.3 ± 0.92 72.3 ± 1.41	80.2 ± 2.3
RM 320 River sediment	41.7 ± 0.8	6	44.3 ± 0.4	42.3 ± 1.6
RM 414 Plankton	4.1 ± 0.1	8		4.0 ± 0.2
RM 422 Cod muscle	0.093	5	0.074 ± 0.092	0.085 ± 0.015

2.3.3 More complicated applications

It is tempting to believe that ID-ICP-MS should be the ideal technique for determination of analytes with more than one stable isotope by ICP-MS, but it is important that the possible existence of polyatomic or isobaric interferences on the isotope pair used for analysis must be considered (an excellent review of interferences in ICP-MS was recently published by Evans and Giglio [15]). Lyon and Fell [16] determined copper in human serum both directly and following separation of the serum by size exclusion chromatography on a G25 Sephadex column using deionised water to load and elute the column. They reported that it was not possible to make an accurate determination of the ^{63}Cu : ^{65}Cu ratio on the diluted serum sample due to the formation of a $^{40}\text{Ar}^{23}\text{Na}$ polyatomic species, but that accurate results were obtained following chromatography. In the case of isotope dilution determinations it is clearly imperative to ensure that the measured ratios reflect the isotopic compositions of the sample, mixture and spike and are not influenced by interferences. This is a straightforward task in general since most elements have known, fixed isotopic ratios.

It is simply a question of verifying that a given ratio lies within the bounds of accuracy once experimental error and mass fractionation are taken into account. The prudent analyst will always measure the natural ratio in a sample rather than use its literature value, in order to avoid errors with radiogenic elements or from unpredicted interferences. Great care must always be exercised when using an isotope dilution method since by its very nature isotope dilution results are often considered to be beyond reproach. It is important that those charged with producing certified reference materials are aware of the possible sources of error with the technique and evaluate isotope dilution data with the same careful scrutiny used for that from other techniques.

Colodner *et al.* [17] used flow injection ID-ICP-MS to determine Pt, Ir and Re in natural waters and sediments in a study aimed at elucidating the behaviour of these elements in the marine environment. They reported detection limits of 5, 6 and 14 pg for Re, Ir and Pt respectively. Anion exchange chromatography was used to avoid isobaric molecular interferences due to rare earth elements (REE) (namely Lu, Yb, Tm and Hf) and achieve a preconcentration (although oxide levels for REE were less than 1% of their parent peaks with the ICP-MS instrument used, REE concentrations were up to 10^4 times higher than those of the analytes. However, this excess was reduced to less than 10 fold following anion exchange chromatography) quantitation was achieved by spiking samples prior to column chromatography with appropriate tracers. Flow injection methodology was used in this case in order to minimise the consumption of sample thereby maximising the pre-concentration factor by eluting the columns into the smallest possible volumes (in fact column eluents were subsequently evaporated down to improve analyte pre-concentration).

As stated earlier, one of the strengths of isotope dilution is that once spike and sample have reached an equilibrium further manipulations do not need to be quantitative since the ratio of spike to sample will not be affected (although if losses are considerable then counting statistics may adversely affect the result). Bearey *et al.* [18] made use of this principle for the determination of Ni in two new NIST standard reference materials (SRM 1515 peach leaves and SRM 1547 apple leaves) and Ni, Mo, Ag and Cd in three candidate reference materials covering a range of soil types and origins. Direct determination of the analytes was precluded due to isobaric interferences with polyatomic ions due to matrix and minor ions (e.g. $^{91}\text{Zr}^{16}\text{O}$ on ^{107}Ag). Firstly, the severity of such polyatomic interferences was estimated by comparing the observed isotope ratio for an analyte in the matrix against its ratio in an elemental standard. When the measured ratio fell outside the estimated experimental error the presence of an isobaric interference was confirmed (such an exercise should be used more generally for ICP-MS and indeed, other instrumental analysis techniques, before abandoning a sensitive choice of analyte signal since the formation of polyatomic ions is sensitive to instrumental conditions and dependant on matrix composition: just because an interference is possible it doesn't mean that it will automatically be severe enough to compromise analytical performance). Separations were then designed to significantly reduce interfering elements without going to the extreme of completely separating the analyte from its matrix, thereby simplifying the analytical procedure considerably. Since isotope dilution methodology was used it was not necessary to achieve quantitative recovery and even day to day batch to batch variations would not affect the quality of the result. Post column ICP-MS isotopic analysis of unspiked samples gave ratios

in agreement with their true values indicating that the chromatography had successfully overcome the interferences. The isobaric interference due to $^{91}\text{Zr}^{16}\text{O}$ was resolved by an electrochemical separation of Ag with a yield of $\approx 70\%$. Excellent agreement was found for Ni in the two certified reference materials whilst no corroborating data was provided for the candidate soil reference materials. This example serves to highlight the potential of this type of analysis. Whilst it is true that much of the initial appeal of ICP-MS lay in its extremely good detection limits, relative freedom from interferences and minimal sample handling requirements, it must not be forgotten that the chemists skills can still be applied to the technique to surmount difficulties encountered in real samples and to boost its performance still further.

A subtle pitfall with the technique was highlighted by Gregoire [19] in a paper dealing with the determination of boron in saline waters. Using a Sciex Elan ICP-MS system, Gregoire noted a matrix induced component to the mass fractionation of boron with $^{11}\text{B}:^{10}\text{B}$ ratios biased to the high side in the presence of high concentrations of concomitant ions (eg the presence of 6 g.l^{-1} sodium results in a ratio 8% higher than the certified value). Since mass fractionation and the determination of the spike isotope ratio used for isotope dilution are both made on aqueous isotopic standard solutions, the matrix induced bias would not normally be compensated for. The possibility of adding such standards to a synthetic blank matrix to mimic the effect is not an ideal solution since it would introduce uncertainties in the observed ratios because of possible contamination problems (it should be noted that such a matrix may well induce signal suppression and therefore a blank contribution could be masked). Gregoire surmounted the problem by using an anion exchange column with a low affinity for sodium to separate the matrix from the analyte. At a recent conference, Gregoire [20] commented that the phenomenon was probably associated with a space charge effect occurring within the ion optics [21]. However the space charge phenomenon has not been reported on in any detail for the VG PlasmaQuad or the other instruments which have recently entered the market place and so it is not possible to comment on the severity of such a problem in these systems, but it would be prudent to evaluate the problem before embarking on any isotope dilution experiments involving light elements.

Campbell *et al.* [22] reported their observation that the time of addition of the isotopic spike (or indeed standard addition) played a critical role in the accuracy of the final result for the determination of mercury in a candidate (now certified) reference material, BCR CRM 422 Cod muscle. If the spike (or addition) was made prior to an overnight digestion period in nitric acid which preceded a microwave digestion stage, then a result was obtained which was in good agreement with that from cold vapour atomic fluorescence spectrometry analysis performed in the same laboratory. However, if external calibration was used or if the spike was added just before microwave digestion then the result was approximately half of its predicted value (despite the use of platinum or thallium internal standards). The same digestion procedure was used for both analytical techniques and it had already been established as giving acceptable results for mercury by the analysis of a range of reference materials. The digestion procedure used produced a solubilization of the sample rather than its mineralisation and these authors attributed the difference in results to a difference in the form of mercury reaching the plasma. Mercury has a high ionisation potential and has therefore a low ionisation efficiency in the plasma. They postulated that the presence of an

organic moiety reduced sensitivity for Hg still further and thus quantitation against inorganic mercury (external calibration) or against an isotopic spike that had not reached equilibrium with the endogenous mercury would give a low result. In cases where the calibrant had equilibrated with the endogenous Hg there would no longer be a difference in response and the correct result would be obtained. It is a commonly held view that all forms of an element entering the plasma are converted to (essentially) mono-positive ions and therefore if spike and sample are in solution equilibrium is not essential since this will be achieved in the plasma: this work suggests that things might not be so straight forward. A piece of confirmatory evidence indirectly supporting Campbell's view was recently reported by Longerich [23]. In an effort to determine the source of a previously unknown interference observed on light REEs he discovered that equimolar quantities of HCl and HClO₄ did not give rise to the same polyatomic interferences, as one would predict. An enhancement was observed in the mass spectra of several oxychlorine species (ClO₂⁺, HClO₂⁺, ClO₃⁺, HClO₃⁺, HClO₄⁺ and H₂ClO₄⁺) in a solution of perchloric acid compared to the spectra observed in hydrochloric acid. Similar phenomena were observed for calcium and magnesium oxychlorine species in perchloric acid, but not in HCl. However, other major rock forming elements did not give enhanced signals from oxychlorine polyatomic atoms. The fact that similar concentrations of chlorine in different speciations gave rise to different polyatomic ions under identical experimental conditions supports the idea that under certain circumstances the form of an analyte entering the plasma can influence its behaviour within the plasma.

2.3.4 Speciation and metabolic studies

As understanding of the importance of the chemical form of a trace element to its biochemical effects (nutritional or toxic) becomes more widespread, the role of isotope dilution will become increasingly important. Stable isotope tracers can be used in two different ways in speciation and (more generally) in metabolic studies: quantitatively, as discussed above, or as labels to identify certain compounds. In this latter role, many metabolic studies may be envisaged (pathway studies, biological half life studies, sequestering etc) and similar types of usage can be imagined for environmental research.

The use of stable isotopes to study various aspects of human and animal metabolism has been pioneered by Janghorbani and Ting. These researchers presented an overview of the challenges posed by this fascinating area of research and illustrated it with a study of the uptake of two stable selenium isotopes in rats [24]. Hydride generation was used to optimize sensitivity for selenium analysis and avoid isobaric interferences. Use of stable selenium isotopes enabled Moser-Veillen *et al.* [25] to undertake a study of the nutritional speciation of selenium in lactating women. Such a study would never have received ethical approval if radioactive tracers had to be used due to consideration of the possible detrimental health effects of low level radiation on mother and new born infant. In a similar study Whittaker *et al.* [26] studied the iron absorption in women to assess the need for iron supplementation during pregnancy. The problem of polyatomic interferences due to the presence of the various ArO species was resolved to acceptable levels by using electrothermal vaporisation as the sample introduction technique. Stable isotope tracers have also been used for Zn bioavailability studies [27] and to determine the life span of red

blood cells *in vitro* in horses using an enriched stable chromium isotope [28].

Isotope dilution will provide a powerful tool in speciation studies in the future. Speciation analysis is a challenging and complex issue. By definition, the speciation analyst is often working with ultra-low concentrations of analyte species since frequently the total elemental concentrations in the parent matrix are very low. The fundamental problem associated with speciation analysis is strongly analogous to the Heisenberg uncertainty principle in sub atomic physics: how can the analyst be certain that in determining the concentration of one analyte species he hasn't disturbed the equilibrium between the forms of the analyte in the matrix ? In the case of airborne or aquatic systems, a careful analyst can be fairly sure that his results reflect the real world conditions, but as soon as speciation of solid materials such as sediment or soil samples is undertaken then Pandora's box has been opened. In order to determine forms of a given element solid samples must invariably be leached, extracted or dissolved and it is certain that these drastic treatments can radically alter the natural species equilibria. Leading researchers in this field such as Ebdon, Rauret, Sanz Medel and Donnard are already posing the idea of operational definitions for solids speciation *ie* the fraction of an element that is determined after a specific chemical treatment. If a stable isotope analog of a species to be determined (either in the same oxidation state in the case of an inorganic spike or incorporated as a label in a metallo-organic compound) can be introduced to the sample and allowed to come to equilibrium then the analyst can see immediately if his sample handling process disturbs the chemical equilibria causing interconversion of chemical forms. Similarly, one has an inbuilt means of quantitation and separative losses are compensated for. Initial studies will no doubt focus on oxidation state chemistry because of the relative ease of producing suitably labelled species and due to the very high cost involved in producing synthetic labelled analogues of organo-metallic compounds. The role of isotope dilution in environmental speciation research is discussed in greater detail by Brown *et al.* elsewhere in this book and is mentioned here for the sake of completeness.

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3.

Detection of sources of error in the determination of Cr in environmental matrices by FAAS and ETAAS

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During the last few years chromium determination in environmental and biological samples has received considerable attention owing to its importance not only as an essential element in living organisms but also to its toxicity even at low concentrations. A considerable number of pollution sources are responsible for the presence, accumulation and mobility of this element in the different environmental compartments.

The chromium content in environmental samples is usually at the trace ($\mu\text{g.g}^{-1}$) or ultratrace (ng.g^{-1}) levels, and thus, an analytical technique of the required sensitivity is necessary. The technique most widely used is Atomic Absorption Spectroscopy with flame (FAAS) or electrothermal atomisation (ETAAS).

Since the late sixties, the determination of chromium by FAAS and ETAAS has been widely studied but there is disagreement with regard to the optimal conditions. Controversial opinions exist about the instrumental conditions recommended to carry out the measurements and about the effects produced by concomitant species in the samples and by the reagents added in the treatment step. This situation demonstrates the difficulties in the analysis of chromium, especially in complex matrices.

The difficulties are more evident when comparing the results obtained from the analyses of a common sample by different laboratories. An exercise organised by the BCR to certify Cr in a calcareous loam soil demonstrated that the spread of the results was too large to be acceptable for certification. No satisfactory explanation was however given [1].

The challenge for analytical chemists is to clarify this situation and requires an in depth review of the literature, followed by a systematic study to detect the sources of error in the analysis of chromium by the spectroscopic techniques mentioned above. An exhaustive study was therefore performed within the BCR programme, and the main aspects are described in this chapter.

3.1 Determination of chromium by FAAS

3.1.1 State of the art

The main problem addressed in the literature with respect to the determination of chromium is the effect of the interferences. All the efforts described are focused on the minimization of these effects by using different types and stoichiometries of flames as well as by addition of different releasers or masking agents. The effect of the oxidation state of chromium and the sensitivity which can be achieved in the determination are other important aspects to be considered.

3.1.1.1 Type of flame

Two types of flame are widely mentioned in the literature, air-acetylene and nitrous oxide-acetylene flames, for the analysis of both environmental and alloy samples, although there is no firm recommendation about the type or composition of flame to be used.

a) Air-acetylene flame

In favour of a fuel-rich luminous or a slightly fuel-rich air-acetylene flame on the verge of luminosity [2-18] is its higher sensitivity (almost a ten-fold increase in comparison to a fuel-lean flame) and attempts are made to suppress interferences by adding different releasers or masking agents. However, Thompson [19] pointed out the lack of reproducibility in calibration when using the luminous air-acetylene flame.

Recommendations for using a fuel-lean air-acetylene flame are made on the basis of its lower interfering effects [19] although other authors pointed out its lower precision [20].

b) Nitrous oxide-acetylene flame

Many authors agree with the ability of the nitrous oxide-acetylene flame to overcome interfering effects [7,19-29] in spite of its lower sensitivity [7,19,21] giving higher detection limits than those obtained with an air-acetylene flame. In some cases however, a higher sensitivity is reported when using this type of flame [22,30].

Interfering effects are not completely removed in the nitrous oxide-acetylene flame [5, 31, 32] and some elements may even cause a higher interference in this flame than that observed in the air-acetylene flame [9]. The interferences depend on the stoichiometry of the flame although this dependence is not as great as that observed in the air-acetylene flame [20].

Another controversial point when using the nitrous oxide-acetylene flame is the addition or not of an ionization buffer such as KCl to decrease chromium ionization. Whereas some authors recommend the addition of potassium [7,19,28], others recommend not only the absence of potassium but also the preparation of calibrant solutions from pure metallic chromium in order to avoid the use of $K_2Cr_2O_7$ [23].

In conclusion, the type of flame and its stoichiometry affect both the sensitivity of the measurement and the effect of interferences. A fuel-lean flame usually decreases the inter-element effects but also decreases the sensitivity, whereas a fuel-rich flame enhances both the sensitivity and the interference effects.

3.1.1.2 Effect of burner height

Another variable which can overcome the interfering effects is the burner height. The measurements at 8-12 mm of burner height are widely recommended, especially when using a fuel-rich air-acetylene flame [2,15,32] but also with different oxidant/fuel ratios in both air-acetylene and nitrous oxide-acetylene flames [12,20,30,37,38]. However, a

clear recommendation could not be drawn from the literature reviewed for the optimum burner height. The measurements of Cr at the higher regions of the flames have been recommended [39,40] to avoid interferences, although cations such as K, Na and Cu may give rise to interferences at this height in an air-acetylene flame [35], whereas measurements at lower regions of the flame (3-5 mm) by both types of flame were also recommended [7,8,22,23,41] although differences in the behaviour of the two oxidation states of chromium in this region were reported [5,21].

3.1.1.3 Instrumental parameters

Other instrumental parameters to be considered, such as the slit-width, the intensity of the lamp and the type of burner have less influence on the chromium determination and these parameters mainly depend on the commercial instrument available. Some recommendations are, however, described in the literature such as the use of a high intensity lamp to obtain better sensitivity [22] and the use of a 3-slot burner which significantly modifies the conditions of the analysis by increasing the reproducibility [19] but also enhances the effect of the interferences [31].

3.1.1.4 Effect of the oxidation state

As far as the behaviour of the different oxidation states of chromium is concerned differences in sensitivity are found in the lowest region of the air-acetylene flame with compounds of Cr(III) and Cr(VI), especially when some anions are present (perchlorates and sulphates) [5,30,33-35]. This difference in sensitivity seems to be negligible in the nitrous oxide-acetylene flame using potassium as ionization buffer [33,35]. Thompson [19] reported differences in relation to the oxidation state in the luminous air-acetylene flame, these differences depending on the concentration level of chromium.

Effects of interferences were shown to be influenced by oxidation state influences [4,21]. However, no difference in the calibration graph between Cr(III) and Cr(VI) was found by other workers with calibrant solutions [14,36] or in spiked real samples [7].

The complexity of the topic is well illustrated by opposite conclusions by Thompson on effects of oxidation state on the use of calibration solutions [19] or spiked real samples [7].

In conclusion, the effect of the oxidation state is still not elucidated and seems to vary widely from one author to another and depending on the conditions used.

3.1.1.5 Interferences in FAAS

The substances described as interferents in FAAS are cationic, anionic or acidic matrices resulting from the sample pretreatment and the concomitant species. The interfering species, the effects observed and the proposed ways of removing interferences for air-acetylene flame are summarized in Table 1.

As can be concluded from this table, the interfering effects are highly variable and in some cases the effects observed are slightly different if the cations are added as chloride or as nitrate [36].

For releaser agents, Ihnat [20] found that the recovery of low levels of chromium is not improved even if the most effective agents are added when this element is analyzed in different synthetic matrices containing cationic and anionic species. Among the releasers tested by this author, NH_4HF_2 , $\text{K}_2\text{S}_2\text{O}_8$, K_2SO_4 , NH_4Cl , Na_2SO_4 , Na_2SO_3 , CaCl_2 , SrCl_2 and AlCl_3 , only NH_4HF_2 and $\text{K}_2\text{S}_2\text{O}_8$ did not cause deterioration of the situation. With real samples, the addition of AlCl_3 gave the highest recovery [20].

Losses of chromium by volatilization when HClO_4 or HCl are added to digest the samples are described as another type of interference, and mixtures of $\text{HNO}_3/\text{H}_2\text{SO}_4$ are recommended for the attacks [47].

A different approach to minimize the effects of the interfering substances is the inclusion of a selective extraction step with a previous oxidation process. Chromium must then be measured in the organic phase as soon as possible because of its instability in this phase [8,37,48,49].

The nitrous oxide-acetylene flame is widely proposed since it removes most of the interferences mentioned above, but some additional interfering effects may occur which are summarized in Table 2.

3.1.1.6 Conclusion

An overall conclusion from the literature review is that the analysis of chromium by FAAS is still controversial and definite recommendations concerning either the type of flame or the effect of the interfering substances are difficult to draw. There are serious obstacles to the achievement of good results in chromium determination in complex matrices at low concentration levels. The main source of error is attributable to matrix interferences, the stoichiometry of the flame being one of the most important parameters associated with these effects.

3.1.2 Systematic study of chromium determination by FAAS

The role of the type of flame, burner height, instrumental parameters, the oxidation state and the interferences in chromium determination need to be clarified. A systematic study was carried out with environmental matrices. The experimental design and the results obtained, together with a critical comparison between them and those found in the literature, are summarized in this section.

3.1.2.1 Type of flame and burner height

The type of flame, the flame stoichiometry and the burner height were studied to achieve the best sensitivity for the determination of low levels of chromium in environmental and biological samples. The different parameters were optimized by means of a modified Simplex approach [52, 53] which considers the variables simultaneously. This method has only been applied once in the optimization of the acetylene and air flows [18] whereas in the literature the variables are generally established in a sequential way.

The response to be optimized was the absorbance reading of a calibrant solution. The Simplex was performed in different working sessions with solutions of Cr(VI) from $\text{K}_2\text{Cr}_2\text{O}_7$ and with two different solutions of Cr(III) , one of them obtained by reducing Cr(VI) and the other prepared from a nitrate salt.

The ranges of the studied variables were: oxidant/fuel ratio 1.5-10 in air-acetylene flame and 0.69-1.93 in nitrous oxide-acetylene flame; burner height 0-15 mm. These experimental ranges excluded the gas ratios with danger of explosion, but considered the composition of the fuel-rich and the fuel-lean flames.

The Simplex was stopped when the improvement in the response was negligible in five consecutive vertices. Seventeen movements were necessary. The conditions which gave the maximum sensitivity are shown in Table 3. All the solutions tested led to the same conditions.

Table 1: Effect of the interfering compounds in air-acetylene flame

INTERFERENT	OBSERVED EFFECT	REMOVAL OF INTERFERENCES
Li	Depressing Cr(III) [44]	-----
Na	Depressing [7,21] only Cr(III) [44]/Enhancing [15]/No effect [36]	2% NH_4ClO_4 [7]/ 4-aminosalicylic acid [15]/ $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame [21]
K	Depressing [21] only Cr(III) [44]/Enhancing [15]/No effect [4,36]	4-aminosalicylic acid [15]/ $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame [21] -----
Cs	Enhancing Cr(III) [44]	-----
Mg	Enhancing [21]/Depressing [4,7,9,15,36]	1% NH_4HF_2 + 0.2% Na_2SO_4 [4]/ 2% alkaline sulphates [9] 2% NH_4ClO_4 [7]/ 1% Na_2SO_4 [36]/ 4-aminosalicylic acid [15] $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame [21]
Ca	Enhancing [21,39]/Enhancing Cr(III) and depressing Cr(VI) [4]/Depressing [7]/No effect [9,36]	1% NH_4HF_2 + 0.2% Na_2SO_4 [4]/ $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame [21]/ 2% NH_4ClO_4 [7]/ Boric acid and KCN [39]
Sr	Depressing [21]/Enhancing [9,39]	2% alkaline sulphates [9]/ $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame [21]/ Boric acid and KCN [39]
Ba	Enhancing [15,21]/Depressing [36,43]/Depressing Cr(VI) and no effect on Cr(III) [4]/No effect [9]	1% NH_4HF_2 + 0.2% Na_2SO_4 [4]/ $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame [21]/ 1% Na_2SO_4 [36]/ KCl as ionization buffer [43]/ 4-aminosalicylic acid [15]
Ti	Depressing [9,21,36]/Enhancing at low concentrations and depressing at high concentrations [5]	0.8% oxine [5]/ 2% alkaline sulphates [9]/ $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame [21]/ 1% Na_2SO_4 [36]
V	Depressing [7]/Enhancing [15]	2% alkaline sulphates [9]/ 4-aminosalicylic acid [15]
Mn	No effect Cr(VI) and depressing Cr(III) [21]/Depressing [14,15,36]/Depressing Cr(VI) and enhancing Cr(III) [4]/Enhancing [9]	4-aminosalicylic acid [15]/ 1% NH_4HF_2 + 0.2% Na_2SO_4 [4]/ $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame [21]/ 1% Na_2SO_4 [36]/ Hexamethylenetetramine [14]/ 2% alkaline sulphates [9]
Fe	Depressing [2,3,4,5,6,7,9,11,13,14,36,39,40]/Enhancing Cr(VI) and depressing Cr(III) [21]	Fe to the standards [2]/ 1% NH_4HF_2 + 0.2% Na_2SO_4 [4]/ $\text{N}_2\text{O}-\text{C}_2\text{H}_2$ flame [21]/ 1% Na_2SO_4 [36]/ Hexamethylenetetramine [14]/ 0.8% oxine [5]/ 2% alkaline sulphates [9]/ 1% $\text{NH}_4\text{OH} \cdot \text{HCl}$ [6]/ 2% NH_4ClO_4 [7]/ Boric acid and KCN [39]/ 2% NH_4Cl [3]

Table 1 (cid)

INTERFERENT	OBSERVED EFFECT	REMOVAL OF INTERFERENCES
Co	Depressing [4,5,9,14,36]	1% NH_4HF_2 + 0.2% Na_2SO_4 [4]/ 0.8% oxine [5]/ 2% alkaline sulphates [9]/ 1% Na_2SO_4 [36]/ Hexametilentetramine [14]
Ni	Depressing [2,4,5,9,14,36,39]/ No effect on Cr(VI) and depressing Cr(III) [21]	Ni to the standards [2]/ 1% NH_4HF_2 + 0.2% Na_2SO_4 [4]/ $\text{N}_2\text{O-C}_2\text{H}_2$ flame [21]/ 1% Na_2SO_4 [36]/ Hexametilentetramine [14]/ 0.8% oxine[5]/2% alkaline sulphates [9]/Boric acid and KCN [39]
Cu	Enhancing [9,21]/Depressing [15,36]/Depressing Cr(VI) and enhancing Cr(III) [4]	1% NH_4HF_2 + 0.2% Na_2SO_4 [4]/ $\text{N}_2\text{O-C}_2\text{H}_2$ flame [21]/ 2% alkaline sulphates [9]/ 4-aminosalicylic acid [15]
Zn	Depressing [4,21]/Enhancing [9]/No effect [36]	1% NH_4HF_2 + 0.2% Na_2SO_4 [4]/ $\text{N}_2\text{O-C}_2\text{H}_2$ flame [21]/ 2% alkaline sulphates [9]
Zr	Depressing [9,40]	2% alkaline sulphates [9]
Mo	Depressing [2,4,9,12,36]	Mo to the standards [2]/ 1% NH_4HF_2 + 0.2% Na_2SO_4 [4]/ 2% alkaline sulphates [9]/ N-cianoacetylacetaldehyde hidrazone [12]/ 1% Na_2SO_4 [36]
Rh	Depressing [12]/No effect [9]	N-cianoacetylacetaldehyde hidrazone [12]
Pd	Depressing [9]	2% alkaline sulphates [9]
Ag	Depressing [36]/Depressing Cr(VI) and enhancing Cr(III) [4]/Enhancing [9]	1% NH_4HF_2 + 0.2% Na_2SO_4 [4]/ 2% alkaline sulphates [9]/ 1% Na_2SO_4 [36]
Cd	Enhancing [4,9,36]	1% NH_4HF_2 + 0.2% Na_2SO_4 [4]/ 2% alkaline sulphates [9]/ 1% Na_2SO_4 [36]
Al	Enhancing in luminous flame and depressing in semi-luminous flame [3,36,42]/Enhancing [10,21,39]/Depressing [4,9]/Enhancing at low concentration and depressing at high concentration [5]	1% NH_4HF_2 + 0.2% Na_2SO_4 [4]/ 2% alkaline sulphates [9]/ 1% Na_2SO_4 [36]/ $\text{N}_2\text{O-C}_2\text{H}_2$ flame [21]/ 2% NH_4Cl [3]/ 0.8% oxine[5]/ Boric acid and KCN [39]
Sn	Depressing Cr(VI) and enhancing Cr(III) [4]/Depressing [10]	1% NH_4HF_2 + 0.2% Na_2SO_4 [4]

Table 1 (ctd)

Pb	Depressing Cr(VI) and no effect Cr(III) [4]/Enhancing [9]/No effect [36]	1% NH_4HF_2 + 0.2% Na_2SO_4 [4]/ 2% alkaline sulphates [9]
Hg	Depressing [36]/Depressing Cr(VI) and enhancing Cr(III) [4]	1% NH_4HF_2 + 0.2% Na_2SO_4 [4]/ 1% Na_2SO_4 [36]
W	Depressing [2,9]/Enhancing [36]/Depressing Cr(VI) and enhancing Cr(III) [4]	W to the standards [2]/ 1% NH_4HF_2 + 0.2% Na_2SO_4 [4]/ 2% alkaline sulphates [9]/ 1% Na_2SO_4 [36]
Bi	Enhancing [4,36]/No effect [9]	1% NH_4HF_2 + 0.2% Na_2SO_4 [4]/ 1% Na_2SO_4 [36]
NO_3^- , NO_2^-	Depressing [39]/ No effect [45]	Boric acid and KCN [39]
PO_4^{3-} , SO_4^{2-}	Depressing [7,39,46]	Boric acid and KCN [39]/ KCN [46]/ 2% NH_4ClO_4 [7]
I ⁻	Enhancing [39]	Boric acid and KCN [39]
AcO^-	No effect [45]	-----

TABLE 1. (cont.)- Effect of the interfering compounds in air-acetylene flame.

INTERFERENT	OBSERVED EFFECT	REMOVAL OF INTERFERENCES
HCl, HNO_3	No effect [4,11,36]	-----
H_2SO_4 , H_3PO_4	Depressing [4,36]	1% NH_4HF_2 + 0.2% Na_2SO_4 [4]/ 1% Na_2SO_4 [36]
HClO_4	Depressing [4]/ No effect [36]	1% NH_4HF_2 + 0.2% Na_2SO_4 [4]
NaOH	No effect	-----
NH_4OH	Depressing [36]	1% Na_2SO_4 [36]

Table 2: Effects of the interfering compounds in nitrous oxide-acetylene flame

INTERFERENT	OBSERVED EFFECTS	REMOVAL OF INTERFERENCES
Fe	Enhancing [23,50]	Fe to the calibrant solutions [23]
Ti	Enhancing [50]/Enhancing only in fuel-rich flame [26]/Depressing [28]	Use of a fuel-lean flame [26]
V,Mo,Al	Enhancing in a fuel-rich flame [26]	Use of a fuel-lean flame [26]
Mn	Enhancing [50]	Addition of 1000 ppm K or Al [50]
Ni	Enhancing [50]/Depressing [32]	Addition of 1000 ppm K or Al [50]
ZnSO ₄	Depressing [27]	-----
HClO ₄ , H ₃ PO ₄	Depressing [31]	-----
H ₂ SO ₄	Enhancing [31]	-----

Table 3: Optimum instrumental conditions for FAAS

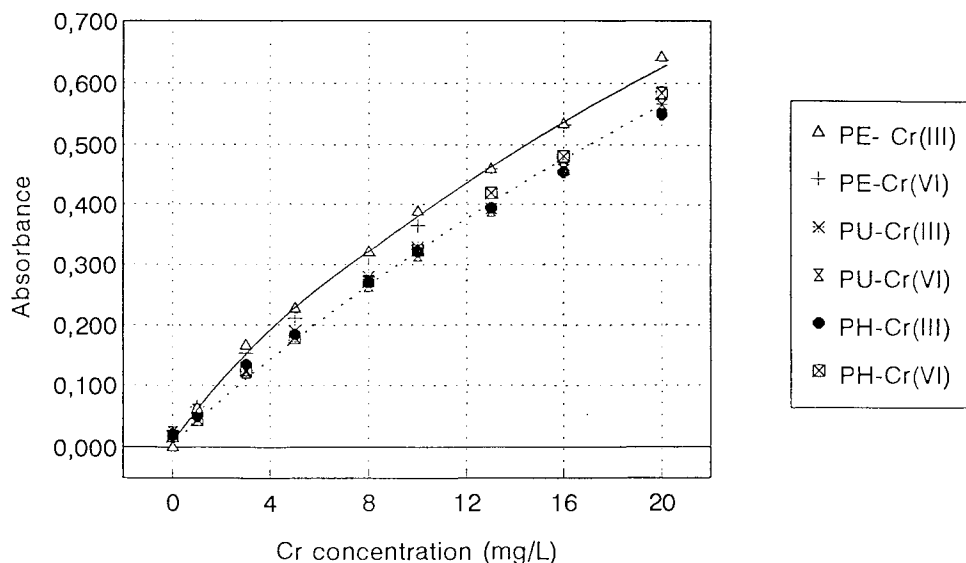
VARIABLE	AIR-C ₂ H ₂ FLAME	N ₂ O-C ₂ H ₂ FLAME
o/f ratio	1.5 (fuel-rich flame)	0.69 (fuel-rich flame)
Burner height (mm)	3	3

The study was carried out with an atomic absorption spectrometer Perkin Elmer model 1100B and the conditions obtained were reproduced in duplicate using two other instruments, a Philips PU 9200X and Philips PU SP900, and with different analysts in order to minimize any systematic error.

The results obtained for oxidant/fuel ratio agree with those found in the literature in relation to the highest sensitivity of the fuel-rich flames [2-18]. As far as the burner height is concerned, 3 mm was found to be the most sensitive region at which interfering effects were not considered.

Other instrumental parameters which do not influence the determination according to the literature, such as slit-width, background correction, different wavelengths and the nebulisation flow rate, were also studied. The conditions established are described in the procedure proposed.

The problem mentioned by Thompson [19] related to the non-linear behaviour of Cr(III) solutions and the lack of reproducibility of the calibration graphs in the luminous air-acetylene flame, assumed by most authors but not confirmed in the literature reviewed, was not observed during our work in any of the three instruments used, for either Cr(VI) or Cr(III) standard solutions, as can be seen from Figure 1.



PE: Perkin-Elmer 1100B / PU: Philips PU9200X / PH: Philips PUSP900

Figure 1: Calibration graphs for Cr(VI) and Cr(III) solutions in fuel-rich air-acetylene flame

3.1.2.2 Interferences

To study the effect of interfering substances, the cations usually found in environmental and biological samples were considered: Na, K, Li, Ca, Mg, Fe, Mn, Ni, V, Mo, Co, Ti, Cu, Cd, Zn, Pb, Hg, As and Al. The interference effect was studied in different Cr:interferent ratios (1:1000, 1:100 and 1:1). The criterion whether or not a substance interferes was deduced from the application of regression lines to compare two series of analytical data [54], which were, in this case, the absorbance readings obtained with and without the addition of interferent. Under the premise of a linear relationship between the two methods, the estimated values for the slope (m) and the intercept (b) were tested against the null hypothesis $m=1$ and $b=0$. If the estimated values differed randomly from 1 and 0 at a predefined significance level (95%), then the methods were equal, *i.e.* the interferent tested had no significant effect on the chromium determination. If the null hypothesis was not fulfilled then the element was considered to interfere. The regression data of this study were those obtained by the Passing-Bablok method [55,56]. The effect of the cations were established for both air-acetylene and nitrous oxide-acetylene flames for the three types of calibrant solutions (Cr(VI), Cr(III) obtained from $K_2Cr_2O_7$, and Cr(III) from nitrate) and the measures were performed in two different working sessions.

The results are summarized in Table 4 where "no effect" means that no interference was observed in a Cr:interference ratio of 1:1000, "slight effect" means that no interference was observed in a Cr:interferent ratio of 1:100, "effect" means that an interfering effect occurred up to a Cr:interferent ratio of 1:1 and "severe effect" means that the interferences were observed in all the concentration ratios studied.

In the air-acetylene flame, ten cations interfered, Ca, Mg and Hg slightly, Mn, V, Mo and Ti up to a 1:100 ratio and Fe, Ni and Co severely. These cationic interferences were always observed by a decreasing effect of the absorbance readings for chromium solutions.

In the nitrous oxide-acetylene flame, it was found that, in contrast to information found in the literature, seventeen of the cations assayed interfered on the measurement of Cr(III) in solutions and nine cations affected the measurements of Cr(VI) in solutions. These cations enhanced the absorbance readings obtained in this flame, except for Ni. These results [57] did not agree with those found in the literature [7, 19-29] where nitrous oxide-acetylene flame was reported to remove all kinds of interference.

The anions studied were chlorides, nitrates or sulphates. The Cr:anion concentration ratio was, as for the cations, 1:1000. With the same criterion as for the cations study, the anions were not found to lead to interferences.

Table 4: Effect of cations upon Cr determination by FAAS

Cation	Air-acetylene Cr(VI) or Cr(III)	N ₂ O-acetylene Cr(III)	N ₂ O-ACETYLENE Cr(VI)
Na	No effect	Enhance	No effect
K	No effect	Enhance	No effect
Li	No effect	Severely enhance	No effect
Ca	Slightly depress	Severely enhance	Slightly enhance
Mg	Slightly depress	Severely enhance	Slightly enhance
Fe	Severely depress	No effect	No effect
Mn	Depress	Severely enhance	Slightly enhance
Ni	Severely depress	Slightly depress	Slightly depress
V	Depress	Severely enhance	Severely enhance
Mo	Depress	Enhance	Enhance
Co	Severely depress	No effect	No effect
Ti	Depress	Severely enhance	Severely enhance
Cu	No effect	Slightly enhance	Slightly enhance
Cd	No effect	Severely enhance	Slightly enhance
Zn	No effect	Severely enhance	No effect
Pb	No effect	Slightly enhance	No effect
Hg	Slightly depress	Enhance	No effect
As	No effect	Severely enhance	No effect
Al	No effect	Severely enhance	No effect

3.1.2.3 *Releaser agents*

For air-acetylene flame the main releaser agents found in the literature, such as NH_4Cl , Na_2SO_4 , $\text{La}(\text{NO}_3)_3$, $\text{Sr}(\text{NO}_3)_2$, 8-hydroxyquinoline (oxine) and $\text{K}_2\text{S}_2\text{O}_8$, were assayed for ten cations which affected Cr determination. For nitrous oxide-acetylene flame, the addition of K was assayed as ionization suppressor. With the same criterion as for the study of the interferences, the releaser agent was considered to be effective when the slope obtained from comparison of the analytical data obtained with a calibration graph without interference and that obtained with the interferent and releaser agent were both close to 1. The regression data considered were those obtained from the Passing-Bablok method [55,56].

The use of potassium for the nitrous oxide-acetylene flame did not improve at all the effect of the interferences observed. The releasers which gave slope values similar to that obtained for the calibration graph for the air-acetylene flame and for all the interfering substances, were 0.5% $\text{K}_2\text{S}_2\text{O}_8$ in some cases and oxine in a most general way. The effect of these releaser agents is shown for Fe, Ni, Co and Mn (Cr:interferent ratio 1:1000) in Figure 2.

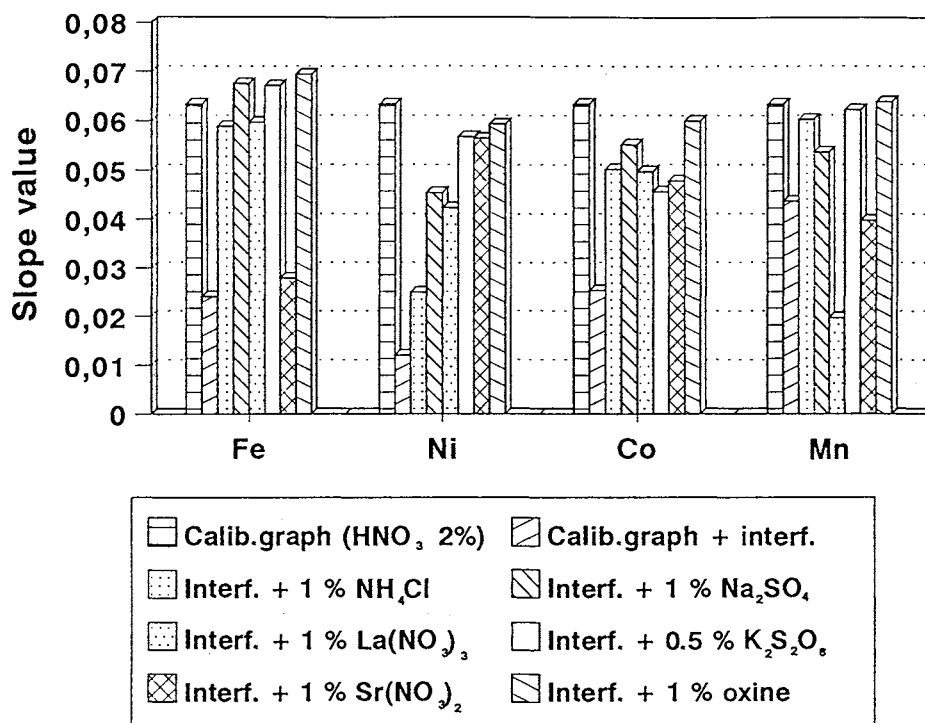


Figure 2: Effect of the releaser agents upon Fe, Ni, Co and Mn in Cr(VI) solutions

3.1.2.4 Conclusions

From the results obtained and the critical comparison with the literature, the overall conclusion is that, in contrast to what it is widely accepted, the use of a nitrous oxide-acetylene flame does not remove the effect of the interferences and gives rise to low sensitivity due to the problem of ionization. Moreover, the use of a fuel-rich air-acetylene flame with the addition of 1 % (w/v) of oxine not only does not demonstrate the occurrence of interferences and is much more sensitive, but is also less expensive and easier to work with.

For all these reasons, we strongly recommend the use of a fuel-rich air-acetylene flame with the addition of oxine when necessary.

3.1.3 Procedure

For the flame atomic absorption measurements, a fuel-rich air-acetylene flame (yellow luminous flame) with an oxidant/fuel ratio of 1.5 is recommended and reading should be at 3 mm of burner height. With regard to other instrumental parameters, 0.2 nm of band-pass, 5-7 ml.min⁻¹ of nebulisation flow rate, a wavelength of 357.9 nm and no deuterium lamp correction, gave the best sensitivity.

For samples solutions, a final medium of 1 % oxine (w/v) is recommended.

3.1.4 Validation of the method

The proposed method was validated by analyzing four sediment certified reference materials (CRM). No other types of CRM were used because Cr levels in biological samples are too low to be determined by flame. The CRM used were: a river sediment from NBS (CRM-1645), a pond sediment from NIES (CRM-n^o2), a river sediment from BCR (CRM-320) and a lake sediment from BCR (CRM-280). The attack of the samples was performed in closed Teflon vessels for the NBS sediment and in open Teflon vessels for the other samples. The acidic digestion was performed by subsequent additions of HNO₃, HClO₄ and HF acids and dissolving the final residue in HNO₃. Different aliquots of the samples were analyzed adding the releaser agent.

The results obtained are shown in Table 5 in which "direct analysis" means that a calibration graph in 2 % HNO₃ was used, and "use of oxine" means that an addition of 1 % oxine to the calibrants was made for the calibration graph. The results are given with 95 % confidence with respect to dried sample.

Table 5: Analysis of sediments (CRM)

Certified Material	Certified value (mg kg ⁻¹)	Direct analysis	1 % oxine
River sediment NBS SRM-1645	29600 ± 2800	27731 ± 1180	28705 ± 2780
Pond sediment NIES CRM-n ^o 2	75 ± 5	54.7 ± 6.0	70.4 ± 2.00
River sediment BCR CRM-320	138 ± 7	121.9 ± 8.4	132.5 ± 6.0
Lake sediment BCR CRM-280	114 ± 4	101.1 ± 2.5	112.4 ± 1.5

From the results obtained it can be concluded that the proposed procedure gives values which were in good agreement with the certified values for all the samples analysed.

3.2 Determination of chromium by ETAAS

3.2.1 State of the art

The determination of chromium by electrothermal atomic absorption spectroscopy (ETAAS) is widely used in different complex matrices especially for biological samples such as urine, blood and serum. It is also applied to certain environmental samples such as water, sediments and sewage sludges.

This determination is still troublesome, the most important possible sources of error being the background correction and the different types of interference described. Other important aspects to consider are the type of graphite furnace, the oxidation state and the type of calibration. In the following sections each of these points will be discussed.

3.2.1.1 *Background correction*

The most common errors in the determination of Cr by ETAAS are background correction errors and it is widely accepted that hardly any real sample can be analysed without background correction [58], although Halls [59] showed that in the case of some vegetable samples the background was extremely low and errors caused by it were small.

The background correction system most frequently used is the deuterium lamp, which is available in most instruments. Some difficulties were described in earlier studies with this corrector, for example the background signal in urine was dependent on the sample volume, the charring temperature and the batch of the tube [60]. These shortcomings were later solved with the development of instruments with better temperature control, and background lamps with greater intensity [61,62] or the use of compromise intensities for the hollow cathode lamp and the background lamp [63,64].

Many authors applied the deuterium background correction system to avoid undesired absorption or light scattering [65,66] for different kinds of samples such as sediments and sewage sludges [67-70], siliceous material [71] and biological samples such as urine [63,64,72,73], human tissues [74] and human milk [61]. However, Sturgeon [75] found severe background interferences when the L'vov platform was used with D₂ lamp correction due to emission from the incandescent platform. Two years later, however, Bettinelli [69] did not find this problem when analyzing sediments. The tungsten-halogen lamp as alternative background correction is also described in the literature [76,77].

More recently, the application of the Zeeman-effect as a background correction system has been introduced [58,72,78-88]. Slavin [58] pointed out that Cr is much better determined with the Zeeman-effect, especially in biological samples which require measurements close to the detection limit, and Dube [84] strongly recommended the Zeeman-effect because the use of deuterium tends to lead to an over-estimation of matrix effects.

Some comparative studies in low ionic strength waters [80] and urine samples [82] showed a good correlation between the two correction systems and no conclusion was drawn on the best suited corrector, although Halls [82] found more background signal and higher detection limits when using the Zeeman-effect.

In conclusion, different background correctors are available at present, but there is no clear recommendation which may be given on the most suited one and the choice seems to be dependent on the matrix of the sample to be analysed.

3.2.1.2 *Effect of the interferences*

Different types of interference are described in the literature: those which arise from sample pretreatment, losses by volatilisation in the ashing step, memory effects after atomisation and interferences caused by the presence of concomitant species.

With regard to the pretreatment step, it is strongly recommended to evaporate the digest until dryness when HClO₄ is added [69,75] in order to remove the acid, which can cause interference in the measuring step, or to add H₂SO₄ together with HNO₃ and HClO₄ to avoid losses of volatile chromium compounds [89].

At the measuring step, there is a serious risk that Cr may be lost at temperatures above 1400 °C during the ashing of the sample [90-92]. Different matrix modifiers allow the use of higher ashing temperatures, hence avoiding losses of Cr. The most widely used is Mg(NO₃)₂ [58,62,69,76,80,86,93] but other species are: alkaline nitrates, Ni and Co [94], ascorbic acid [95], NH₄H₂PO₄ [96], Na₂WO₄ and Na₂MoO₄ [97] and Triton [82,88].

Different mechanisms have been proposed for the removal of memory effects caused by carbides which were not completely dissociated during atomisation, leading to low results [98,99]: a purge with a mixture of Cl_2/N_2 (1:5) after atomisation [99], the addition of CuCl_2 after the ashing step [100] and the addition of a cleaning step at a higher temperature after atomisation, which was widely used [59,72,73,82,84,85,89,94], although Paschal [88] did not find any memory effects even without a cleaning step. However, the formation of carbides after reduction of Cr_2O_3 [91,98-102] was not supported by Arpadjan [97]. The interfering effects of the concomitant species in the samples and the ways to remove them are summarized in Table 6.

3.2.1.3 *Type of graphite furnace and use of L'vov platform*

The use of pyrolytically-coated tubes is widely accepted [59-61,63,73,74,77,78,84,88,90-92,104,107,108,110] due to the higher sensitivity that can be achieved [59,60,73,92,108,110] which is attributed to the higher speed of vaporisation [91], greater precision [73,92], lower memory effects [74,90,107] and the fact that they allow higher number of firings than the uncoated tubes [77]. However, Veillon [60] found higher background signals when using pyrolytic tubes. The use of uncoated graphite tubes is also described in spite of their lower sensitivity [63,65,71,89,103] since the results found are more reproducible [89].

Other type of tubes, such as W or Zr-coated tubes [92], Ta-coated tubes [106] and totally pyrolytically coated tubes [109], have the characteristics of a longer tube life [92, 109], a higher sensitivity [106,109] and a lesser effect of the interferences [92], but they are not commonly used. The use of the L'vov platform is recommended in most cases [70,79-81,86,93,95,96] although some problems of background [59,75] were found.

In conclusion, the use of pyrolytically-coated tubes and L'vov platform seems to be the most general choice for Cr determination.

3.2.1.4 *Effect of the oxidation state*

In agreement with the atomisation mechanism which leads to the formation of Cr_2O , irrespective of the original chromium compound [91,98-102], various authors did not find any difference in sensitivity between the two oxidation states of chromium, Cr(III) and Cr(VI), [61, 94, 103, 105]. The recovery of chromium in spiked samples of seawater [83] and urine [84] was also found to be independent of the oxidation state.

However, Arpadjan [97] found that the losses of chromium during the ashing step were higher for Cr(VI) than for Cr(III) compounds.

3.2.1.5 *Calibration mode*

Another point of discussion in the literature is the correct calibration mode for the analysis. Whereas many authors pointed out that the calibration graph gave results similar to those found by the standard addition method in different matrices such as urine [63, 84], low ionic strength waters [80], tissues [74,86] and sludges [70], other authors found it necessary to use the standard addition method in plant materials [95], urine samples [72,73] and serum [62].

3.2.1.6 *Conclusions*

From the literature reviewed it seems clear that pyrolytically-coated tubes and in some cases the use of the L'vov platform is recommended for Cr determination, but other instrumental conditions such as background correction and the ways to remove interferences or the calibration mode are still controversial points which need further investigation.

Table 6: Effect of the interfering compounds in ETAAS.

Interferent	Observed effect	Removal of interferences
Na	No effect [65,68,80,103]/Enhancing as nitrate [77,104]/Depressing in HCl [104]	Less effect in pyrolytic or Zr-coated tubes [92]/ Double atomisation [103]
K	No effect [65,103]/Enhancing at $T_{atom} > 2400^{\circ}\text{C}$ [64]/Depressing as nitrate [68,77]	Double atomisation [103]
Mg	No effect [68,80,91,103]/Depressing as nitrate and enhancing as chloride [77]	Less effect in pyrolytic or Zr-coated tubes [92]
Ca	No effect [65,80,103]/Enhancing as nitrate [77,104]/Depressing as chloride [68,104,105]	Less effect in pyrolytic or Zr-coated tubes [92]/ EDTA [105]
Sr	Depressing as chloride [105]	EDTA [105]
Ba	Enhancing as nitrate [77]/Depressing as chloride [105]	EDTA [105]
Fe	No effect [103]/Enhancing as nitrate [77,104]/Depressing as chloride [68,77,105,106]	Less effect in pyrolytic or Zr-coated tubes [92]/ EDTA [105]
Cu	No effect [103]/Depressing [77,105]	Double atomisation [103]/EDTA[105]
HNO ₃	No effect [66]/Enhancing [106]/Depressing [74,77,92,107]	Addition of acid to standard solutions [74,77,107]
HClO ₄	Depressing [77,106]	Addition of acid to standard solutions [77]
H ₂ SO ₄	Enhancing [94,95]	----

3.2.2 Systematic study of chromium determination by ETAAS

As for the FAAS technique, a systematic study was carried out, paying special attention to those points which were not clear in the literature, such as the instrumental conditions and the effect of the interferences.

3.2.2.1 Instrumental conditions

Different instrumental conditions such as slit-width, lamp intensity, inert gas (N₂ or Ar), interrupted flow mode during atomisation, type of signal (peak area or peak height), sample volume, background correction (use of tungsten lamp) and type of graphite tube, were studied in order to identify the variables which gave the better sensitivity. From sequential assays in different working sessions at concentrations ranging from 10 to 500 $\mu\text{g.l}^{-1}$ Cr, the variables mentioned above were established with the values shown in Table 7 using a Perkin-Elmer instrument. Taking into account the size of the graphite tubes in this instrument compared with those used in other commercial instruments, it should be considered that these established conditions may be slightly different for each type of matrix and for each type of instrument.

The Simplex approach [52,53] was used to establish the continuous variables, which in this case were the different temperatures and the ramp and hold times for each step of the furnace program. The response considered for Simplex application was the slope value obtained with four calibrant solutions of Cr(VI) from 2 to 30 $\mu\text{g.l}^{-1}$. The ranges of variables studied were those reported in the literature:

Drying : Temp.: 90 - 160°C; Ramp time: 5 - 25 s; Hold time: 10 - 30 s

Ashing: Temp.: 1000 - 1600 °C; Ramp time: 5 - 25 s; Hold time: 10 - 30 s

Atomizing: Temp.: 2300-2700 °C; Ramp time: 1 - 5 s ; Hold time: 2 - 10 s

After 38 movements of the Simplex, the improvement in the slope value was negligible and thus, the optimum conditions shown in Table 8 were obtained.

Table 7: Optimum instrumental conditions for ETAAS technique.

Parameter	Value
Wavelength (nm)	357.9
Type of graphite tube	Pyrolytically-coated
Type of signal	Peak area
Inert gas	Ar
Background correction	On
Flow mode (atomisation)	Stopped flow
Lamp intensity (mA)	10
Slit-width (nm)	0.7
Sample volume (μ l)	20

Table 8: Optimum graphite furnace program

STEP	T (°C)	t _r (s)	t _b (s)
Drying	140	18	19
Ashing	1300	5	15
Atomizing*	2400	3	10
Cleaning	2600	2	4

* stopped flow of inert gas

The temperature and time programs established with this system take into account that Cr may be lost at ashing temperatures above 1400 °C [90-92].

3.2.2.2 Interferences

For this technique the effects of different matrices with Cr contents measurable by ETAAS, such as synthetic waters and biological matrices, were studied.

The interferences were studied for three spiked synthetic water samples of increasing hardness, by means of recovery at two concentration levels, 6 and 24 μ g.l⁻¹ Cr(VI). The calculations were performed with reference to a calibration graph in 2 % HNO₃.

This direct analysis gave very high recovery values, especially for the lowest concentration, and thus, different matrix modifiers and the L'vov platform were tested in order to remove the interfering effects. With respect to matrix modifiers the addition of 20 μl of 1 % NH_4NO_3 [94], 0.4 % $\text{Mg}(\text{NO}_3)_2$ [58,62,69,76,80,86,93] 0.25 and 2.5 % Triton X-100 [82,88], 1 % GeO_2 and 1 $\text{g}\cdot\text{l}^{-1}\text{Pd}$ [111] were assayed. All the modifiers were tested with a pyrolytically-coated tube, as well as with the L'vov platform. All the assays were performed in two different working sessions. As can be concluded from Figure 3, the best recovery values were obtained when using the L'vov platform, as widely recommended in the literature. The same results were found at the two levels of concentration assayed, and the addition of the matrix modifiers mentioned herein did not improve the recovery values found.

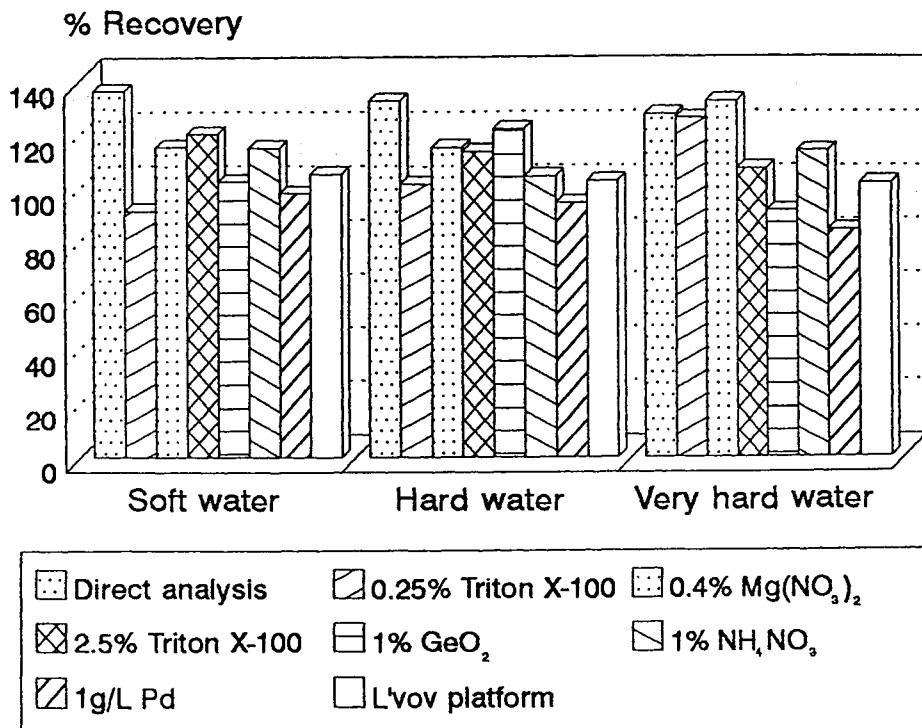


Figure 3: Effect of matrix modifiers and L'vov platform upon spiked water samples ($6 \mu\text{g}\cdot\text{l}^{-1}$)

Chromium was determined in urine samples using two different instruments, a Perkin-Elmer atomic absorption spectrometer with tungsten lamp as background correction and a Varian atomic absorption spectrometer equipped for Zeeman-effect. As described previously [112] the interferences detected were of physical origin, due to the high viscosity of the sample and the sample was thus diluted and a preliminary ashing step at 700°C was included in the program of the graphite furnace. Under these conditions, the chromium content in two NIST certified reference material (CRM-2670) freeze-dried samples (normal and spiked urine "elevated level") were determined using calibration curve as well as standard additions methods. The dried material was reconstituted with 20 ml of double deionized water, and analysed on the same day. The results obtained are shown in Table 9.

Table 9: Analysis of NIST CRM-2670.

Back-ground corrector	NIST value (mg.l ⁻¹)	Values found Calibr.curve (mg.l ⁻¹)	Values found Standard addition (mg.l ⁻¹)
PE instr.	(0.013) informative	0.010 ± 0.001*	0.012 ± 0.001*
VS instr.		0.010 ± 0.002	0.012 ± 0.002
PE instr.	0.085 ± 0.006	0.080 ± 0.003	0.083 ± 0.005
VS instr.		0.089 ± 0.001	0.082 ± 0.002

* value lower than the quantification limit.

As can be seen from the results, the calibration curve and the standard addition methods did not give significant differences, and thus the calibration curve of Cr(VI) in 2 % HNO₃ is proposed.

The PE instrument with tungsten lamp correction is only applicable when the concentration is high enough, *i.e.* for toxic levels of Cr in urine, but no conclusion can be drawn in relation to the best background correction system. Further investigation in this aspect is necessary.

3.2.3 Procedure

For ETAAS technique, the use of the L'vov platform with the temperature program shown in Table 8 is recommended for the analysis of inorganic matrices, such as fresh water with the Perkin-Elmer instrument. The remaining instrumental conditions are shown in Table 7.

Biological matrices should be analysed using a slightly modified graphite furnace program, adding a preliminary ashing step at 700 °C.

3.2.4 Validation of the method

The proposed method was validated by the analysis of three certified reference materials: a mussel tissue from BCR (CRM-278), a dogfish muscle from NRCC (Dorm-1) and a plankton from BCR (CRM-414). The attack of the samples was performed following the Canadian DOE method for the determination of trace metals in fish [113] slightly modified for plankton with the addition of different aliquots of HF and HClO₄.

The results obtained with a 95 % of confidence for five analyses are shown in Figure 4a, b and c. It can be concluded that the method proposed is accurate for the Perkin-Elmer instrument used, but conditions should be optimized for different instruments. Both calibration graph and standard addition methods gave good and comparable results.

3.3 Conclusions

For the FAAS technique it has been clearly shown from the literature reviewed and the experimental work carried out that Cr determination is strongly affected by the interferences. In our experience, the use of a fuel-rich air-acetylene flame with the addition of a releaser agent leads to reliable results, whereas the use of the widely recommended nitrous oxide-acetylene flame has shown enhancing effects for almost all of the cations assayed. From all the compounds tested as releaser agents in air-acetylene flame, 1 % oxine gave the best results.

For the ETAAS technique, the conditions are more dependent on the sample matrix and the characteristics of the instrument used. The recommendations found in the literature for pyrolytically-coated tubes and in some cases the use of the L'vov platform had been confirmed in this study.

With the optimum conditions for each of the techniques, the detection limits achieved were 0.04 mg.l^{-1} Cr for FAAS and $0.34 \text{ }\mu\text{g.l}^{-1}$ for ETAAS, and the precision of the methods were 4.3 % (at 0.4 mg.l^{-1} Cr) and 3.7 % (at 4 mg.l^{-1} Cr) for FAAS, and 7.5 % (at $6 \text{ }\mu\text{g.l}^{-1}$) and 3.3 % (at $24 \text{ }\mu\text{g.l}^{-1}$) for ETAAS.

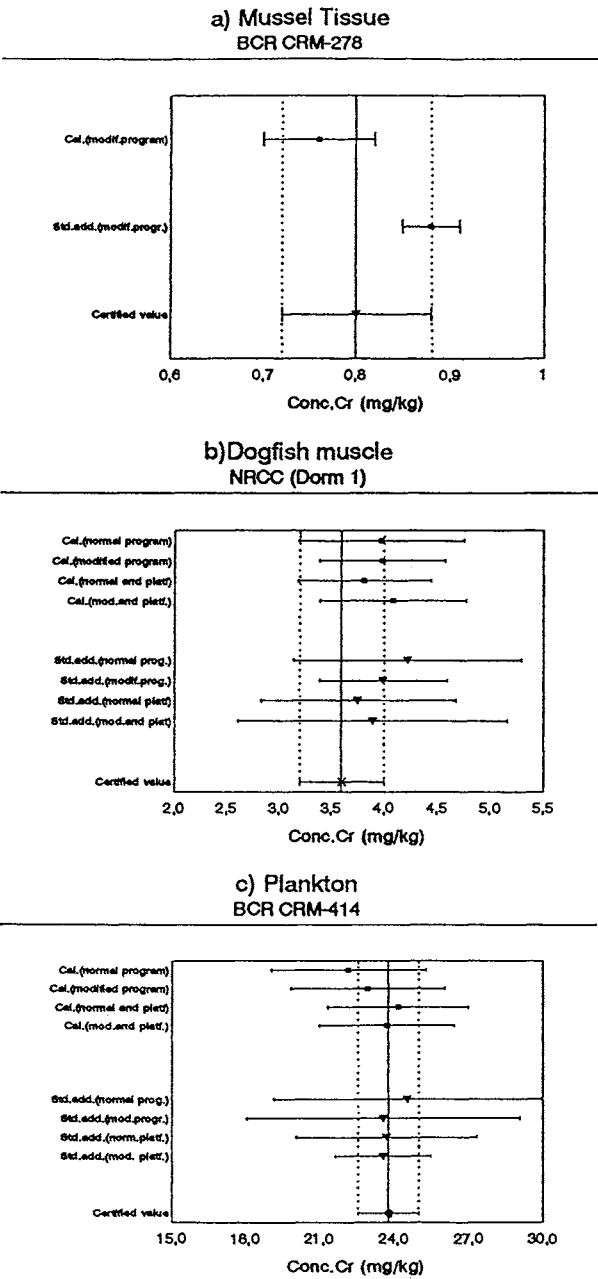


Figure 4: Analysis of different certified reference materials by ETAAS

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4.

Analysis of environmental and biological samples by atomic spectroscopic methods

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The rapid and constant development of human activities increases the need for analytical chemistry, which enables important decisions to be made daily in different fields such as industry, environment or health. These developments affect directly the quality of our daily life.

A few years ago, the analyst's main concern was to perform determinations under the most appropriate conditions and with control of matrix interferences. However, prior to any measurement, there are two fundamentally important stages: sampling and sample preparation, which are too often overlooked in the quality control of environmental and biological analysis. It would be incorrect to say that these two stages were neglected in the past; most analysts were well aware of their importance. Despite significant progress in instrumentation, the quality of the results did not follow the same trend. It appeared necessary to look beyond the instrumentation and it became increasingly obvious that important errors were mostly associated with sample pretreatment stages. Research trends have focused on these critical steps. As a result, analytical chemists are now much better prepared than previously to develop new methods or to control their validity. Sample preparation and development of methods have now become a growing field along with instrumental improvements.

This chapter describes the different sample preparation techniques used prior to atomic spectroscopic analysis and gives an overview of interferences likely to occur in the analysis of environmental and biological samples. The use of the principal atomic spectroscopic techniques and their application to environmental analysis will be discussed, with emphasis on problems associated with preparation of different types of environmental samples.

4.1 Preparation of samples

Environmental or biological samples may be divided into those which are already in solution or liquid state (water, blood, serum, urine *etc.*), and solid samples (soils, sediments, plants, animal tissues *etc.*). Solid samples may contain a high proportion of organic matter (plants, animal tissues) or have a more mineral composition (soils, sediments). For routine analysis by spectrochemical techniques samples are required to be in a liquid form and, hence, solid samples must generally be converted into a solution by an adequate dissolution method.

4.1.1 Liquid samples

Solutions can generally be introduced directly for analysis and without any prior treatment. The risk of contamination increases with decreasing analyte concentration; all vessels to be used have thus to be cleaned, rinsed and then soaked overnight in 1-10% (v/v) nitric acid. All vessels should be then rinsed in high purity deionized water. In order to minimize analyte losses by adsorption of metal ions on the vessel or on the suspended particles, the collected samples can be stored for a short time in a refrigerator, and for longer periods in a deep freezer. For the same purpose, aqueous solutions are generally acidified (< pH 1.5, nitric acid).

4.1.2 Solid samples

Many types of solid environmental samples are passed into an aqueous solution after a dry ashing or a wet digestion.

4.1.2.1 Dry ashing methods

Dry oxidation or ashing may be used to remove organic matter from samples. The sample is weighed in a suitable crucible (generally platinum), heated for several hours at 400-500 °C in a muffle furnace, and the residue is dissolved in an appropriate acid. The method is simple and large sample series may be treated at the same time. However, the dry ashing procedure cannot be applied if volatile elements (*e.g.* Hg, As, Se) are to be determined since they may volatilize during the ashing process. In these cases, oxidants may sometimes be used as ashing aids in order to speed-up the ashing and prevent the volatilization of analytes. Commonly used ashing aids are magnesium oxide and magnesium nitrate.

Another likely cause of losses during dry ashing is the retention of the analyte by some of the solid matter present in the system. The solid matter available for this reaction is generally the material of the ashing vessel and the constituents of the ash of the sample itself. The choice of the adequate crucible is therefore of prime importance: the most important retention losses have been reported for silica and porcelain vessels, but they vary with many factors. The most appropriate material for dry ashing methods is platinum.

4.1.2.2 *Wet digestion methods*

The majority of wet digestion methods involve different combinations of five acids (nitric, sulphuric, perchloric, hydrochloric, hydrofluoric) and hydrogen peroxide. Nitric acid, boiling at about 120 °C, is the most widely used primary oxidant for the destruction of organic matter. It is commonly used in the presence of sulphuric acid, which partially degrades the more resistant material and also serves to raise the boiling point of the mixture, or with perchloric acid, which continues the oxidation after the nitric acid has been removed. Because occasional explosions with perchloric acid may occur, its use is generally avoided. Oxidations with hydrogen peroxide in acid mixtures containing sulphuric acid are based on *in situ* involved permono sulphuric acid [1]. Combined with the dehydrating action of sulphuric acid, this reagent rapidly degrades many organic materials. Mixtures with hydrochloric acid are used generally for samples with prevailing inorganic matrices, and combinations with hydrofluoric acid are used to decompose silicates, which are insoluble in the other acids.

Acid digestions are usually carried out either in glass or Teflon vessels. Pressure dissolution is essentially a wet digestion procedure which is performed under pressure. With this technique, the loss of volatile elements is avoided and the decomposition of more complex matrices is possible. The limiting factor of pressure digestion in "bombs" is the small amount of organic matter which can be treated.

In general, the temperatures involved in wet oxidation methods are very much lower than in dry ashing. The volatilization losses or retentions caused by reaction between the analyte and vessel are then much less frequent. However, possible coprecipitations of the analytes with a precipitate formed in the digestion mixture may sometimes occur. The best known example is the coprecipitation of lead on calcium sulphate precipitates formed when a sample high in calcium is digested with a mixture containing sulphuric acid.

The most recent method of wet digestion employs microwaves as the energy source. The main advantages of microwave digestion are speed, efficiency of decomposition for difficult to solubilize samples, and the possibility of automation. This technique has been shown to be suitable for trace element determinations in a wide variety of matrices; in some cases, however, care has to be taken when *e.g.* organic compounds have to be digested [2].

4.1.2.3 *Direct analysis of solids and solid slurried samples*

There is a growing interest in the determination of elements in solid samples by atomic spectroscopy without carrying out a dissolution step, in order to avoid contamination and losses during preparation of the sample. This approach cannot directly be used in flame AAS (insufficient dissociation of solid particles in the flame with relatively low temperature) and in ICP-AES (physical and chemical interferences due to differences in transport and dissociation phenomena between the solid slurry samples and solutions used for the calibration), but it is particularly convenient for graphite furnace AAS and when only small amounts of sample are available. However, problems may arise because of unrepresentative sub-sampling and enhanced interferences compared with the analysis of solutions.

These and other problems have been discussed in a comprehensive review by Langmyhr and Wibetoe [3]. Several specially designed devices for direct analysis of solid samples using classical AAS have been recently used: graphite cups [4], probes [5], boats [6], specially designed tubes [7] and tube-platform systems [8]. However, direct analysis of solids by electrothermal atomic absorption spectrometry (ETAAS) is initially handicapped due to some restrictive factors not present in the analysis of dissolved samples:

1. The greatest problem originates from sample heterogeneity which requires a substantial effort to obtain representative sub-samples.
2. The determination of relatively high analyte concentrations is limited by the minimum representative sample mass that can be introduced into the atomizer.
3. One of the major difficulties associated with solid sampling concerns the availability of appropriate calibrant of similar composition to the samples analyzed.
4. For multi-element analysis this technique is particularly time-consuming compared to actual analysis of solutions.
5. The interference effects observed with solid sampling are greater compared to the dissolved samples whose matrix is simplified as a result of the mineralization.
6. The good contact between the analyte and the graphite surface of the furnace, necessary for reproducible heat transfer from platform to analyte and also for the possible analyte reduction by the graphite prior to the atomization, is not achieved as satisfactorily as in the case of solutions.
7. The use of matrix modifiers, often required to ensure the efficiency of platform techniques, is problematic.
8. Sample introduction into the atomizer is less convenient compared to the dissolved sample.

For these reasons, the precision obtained by solid sampling is generally less than that obtained with solution analysis. Nevertheless, many researchers consider that solid sampling facilitates analysis in some specific cases and may lead to consistent results.

In 1974, Brady *et al.* [9,10] proposed an interesting method of solid sampling, the dispensing of water-suspended powdered sample into the atomizer, using a micropipette. At the present time, this alternative of introducing solid material as either a suspension or a slurry appears to be the best approach to overcome some of the difficulties associated with the sampling of solids [11-14]. The recent evolution of ETAAS using platforms, autosamplers and adequate signal processing contributes largely to routine applications of this solid sampling alternative. Studies have also been extended to the use of chemical modifiers to minimize the effects of the matrix components [15,16]. The powdered samples are generally suspended in demineralized water and stirred periodically (magnetic stirring or ultrasonic mixing) just before sampling by the autosampler capillary to avoid sedimentation of the particles, which may result in unrepresentative sampling. To overcome sedimentation in the water suspended samples, Littlejohn *et al.* [17] prepared stable slurries with a thickening agent consisting of acrylic acid polymers (Viscalex, Allied Colloids). For the same purpose, Hoenig *et al.* have employed glycerol [16,18].

With regard to the representative sampling of the slurry, it is clear that the presence of large particles in the sample is the most critical factor in the analysis. Dispensing of the solid by the autosampler capillary may be biased by the heterogeneity of the slurry. The number of particles contained in very small samples can be easily evaluated from the size distribution. For example, for 5 μg of a typical silty sediment, the number of particles can vary from several dozen for particle sizes between 16 and 32 μm to several hundred thousands for particle sizes less than 4 μm . The importance of an intensive grinding of the sample prior the analysis is thus obvious [18].

At present, slurry sampling-ETAAS is generally used for environmental purposes (soils, sediments, atmospheric particles, ground plant samples, lyophilized animal tissues, suspended solids collected in natural waters); some industrial applications of this technique can be also investigated.

4.1.3 Usual procedures

4.1.3.1 Plants

Amongst all the procedures known for the mineralization of plant samples, only a method able to dissolve the silica avoids problems due to retention losses of trace elements by the insoluble silica residues; these losses always occur if a dry ashing procedure followed by a simple acid dissolution of the ashes is applied. Thus, the often ignored undesirable effects are eliminated by a more adequate dissolution of the ashes. For this purpose, the "Méthode du C.I.I." (Comité Inter-Instituts des Techniques Analytiques) ensures acceptable recoveries. In this method the ashing is carried out at 450 °C and the silica is removed by a hydrofluoric and nitric acid treatment [19]. This method was tested with environmental certified reference materials (CRMs) and proven to be successful for the determination of major (Ca, K, Mg, Na, P), minor (Fe, Mn) and trace (Cd, Co, Cr, Cu, Mo, Ni, Pb, Sb, Tl, V, Zn) elements; it results essentially in a total decomposition of the sample. Of course, danger of losses by volatilization of certain elements always exists with such a method. The analysis of mercury, selenium and arsenic, for example, cannot be achieved using a dry oxidation procedure. In this case, a wet digestion circumvents this problem as the metals concerned may be dissolved *e.g.* a mixture of nitric and sulphuric acids combined with hydrogen peroxide has been shown to be suitable for plant analysis [20].

For the determination of mercury in plant material, the addition of nitric acid can be omitted; concentrated sulphuric acid is then used only.

4.1.3.2 Animal tissues

Because of the absence of silica in animal tissues, a simple dry ashing procedure followed by a nitric acid and hydrogen peroxide dissolution of the ashes, is generally sufficient. The method was tested with success for a large variety of samples : muscles, organs and fish meal, with satisfactory recoveries for Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn.

Wet digestion procedures may also be used, particularly for the determination of volatile elements. For mercury, the mineralization procedure described for plants can be used. In the case of selenium or arsenic, the most appropriate procedure is the high pressure acid digestion performed in teflon lined bombs which, by maintaining strong acids at temperatures well above their boiling point, accelerates the digestion process in comparison to atmospheric pressure digestion in open vessels; this permits a complete dissolution of samples without risking losses of analytes.

Many organic materials can be decomposed satisfactorily in digestion bombs but careful attention must be given to the composition of the sample matrix and to possible explosive reactions with the digestion media. In all cases the size of the sample and the amount of oxidant used must be carefully controlled. For nitric acid bomb digestions of organic samples, the mass of sample intake must generally not exceed 0.1 g whereas the amount of concentrated nitric acid added to this charge has to be in the range of 2.5 to 3.0 ml.

4.1.3.3 *Soils, sediments and particulate matter*

Simple mineralization procedures are generally used to determine the mobility of elements *e.g.* in environmental and agricultural studies. These methods, called "strong attacks", use mixtures of strong mineral acids, except hydrofluoric acid. In comparison to the "total attacks" (using mixtures with hydrofluoric acid which ensures dissolution and elimination of silica), recoveries of the strong attacks may be slightly poorer, particularly for elements difficult to solubilize, associated for example to silicates.

For certain analyses where the total analyte content must be known, the direct analysis of the solid sample by ETAAS may be performed (slurry sampling) instead of using a complex mineralization procedure. Good results are obtained only if the slurry is stirred periodically, just before sampling by the autosampler capillary. This mixing may be done using a mini-stirrer or an ultrasonic probe, both operated manually. However, this alternative is not compatible with the concept of a complete automation of the procedure and robotization of the mixing system may be adapted to the spectrometric system [21].

Electrothermal programs and chemical modifiers used for ETAAS slurry analysis are generally similar to those employed for analysis of solutions. The frequency of analysis is the same as with solutions and in any case higher compared to actual solid sampling, which requires sample weighing for each measurement. The rapidity of the whole procedure thus easily allows repetitive analysis. Consequently, the analysis of slurried samples provides additional advantages compared to direct solid sampling: compatibility of analysis with conventional ETAAS devices with the same speed of analysis as for solutions, the possibility to dilute slurries or to dispense variable sample volumes, straightforward addition of chemical modifiers and the possibility of complete automation and sequential multi-element analysis on the same sample.

4.2 Plasma atomic emission spectrometry

4.2.1 *Basic principles and instrumentation*

In plasma atomic emission spectrometry the sample, generally in liquid form, is introduced through a nebulization device (pneumatic or ultrasonic) into a plasma source, where it is evaporated and dissociated into free atoms and ions, and further additional energy is supplied to enable excitation to higher energy states. A plasma is a highly ionized gas; its high temperature and the dissociation of the analyte compounds to atoms and ions and their excitation are produced by collision with other particles, mainly with free electrons. The excited state is unstable and the atom or atomic ion loses its excess energy either by collision with other particles or by radiative transition to a lower energy level. The resulting radiation is called spontaneous emission. The atomic emission spectroscopic (AES) methods are based on these emission spectra, which are very complex compared to the absorption spectra. Therefore, an atomic emission spectrometer requires an optical bank with good resolution and the possibility for background and inter-element correction.

The wavelengths of the emission lines are characteristic of the elements present in the plasma source. The detection of radiation at particular wavelengths allows a qualitative analysis of the sample and the measurement of the intensities at these wavelengths gives rise to the quantitative determination of the analytes. Most analytical plasma sources are electrical gas discharges at atmospheric pressure, usually in argon. The temperatures attained in a plasma source are higher than 5000 °C in the viewing zone.

Each spectrometer for sequential (or multi-element) plasma AES measurements is equipped with a monochromator (or polychromator) for adequate wavelength selection and to collect as much light as possible from a selected spectrum area in the radiation source.

A complete plasma atomic emission instrument consists of two main units: the signal generator and the signal processor. The signal generator is represented by the sample introduction system (autosampler, pump, desolvation device, nebulizer *etc.*) and the plasma source. The signal processor comprises the optics and a unit for data acquisition and processing. Various function and parameters of the instrument are now piloted by a microcomputer and the determinations can be carried out automatically according to a preset analytical programme.

4.2.2 Interferences

Interference effects in plasma AES comprise transport interferences (generally nebulization), chemical interferences, ionization interferences and spectral interferences. The degree of interference varies from one instrument to another. However, the most significant impediments to the effective use of any emission spectroscopy equipment are spectral interferences.

4.2.2.1 Transport interferences

Transport interferences are observed if the amount of the sample nebulized varies considerably as a function of time. They may be caused by matrix salts or organic compounds and solvents of different viscosity, surface tension or density. This may also occur to some extent for solutions with high mineral acid concentrations, particularly when using ultrasonic nebulizers. Moreover, memory effects may occur if long tubes and large vessel surfaces are used in the nebulization and desolvation systems, but these effects can be easily avoided and controlled.

4.2.2.2 Chemical interferences

Due to the high plasma temperature, long residence times and the inert atmosphere in the source, chemical interferences caused by an inefficient sample dissociation or by the formation of thermally stable compounds or radicals, are uncommon if an adequate control of main parameters is ensured.

4.2.2.3 Ionization interferences

Easily ionizable elements, such as alkali and alkaline earth elements, may alter the intensities of the emission lines of the analyte. This is a serious problem encountered in direct current and microwave induced plasmas (DCP, MIP), but the effects are minor in the case of inductively coupled plasma (ICP).

4.2.2.4 Spectral interferences

Spectral interferences are observed in every emission method. These interferences are most important in ICP because emission lines that may be expected to be weak or nonexistent in other sources such as flames, are quite intense. All spectral interferences originate from line and continuum spectra of atomic and molecular species present in the source and also from the inherent argon spectrum. They can be classified into four principal groups.

1. Spectral overlap occurs when the monochromator of the spectrometer is not capable separating the analyte line from the interfering line. In this case, the automated interelement correction can be used by introducing a previously determined interelement correction factor into the analytical program. With instruments offering unlimited selection of analyte wavelength, these problems may also be avoided by selecting an alternative analyte line that does not exhibit the interference.
2. A broadened line wing of a matrix element in the vicinity of the analyte line may cause spectral interference by partial overlapping the analyte line. This interference may be avoided by moving to another interference-free line or in some cases by using adequate background correction. The selection of a background correction technique depends on the shape of the background emission which can be flat, linearly sloped, curved or structured.
3. Spectral continuum interference may be caused by one of the matrix components emitting a continuum spectrum at the analyte wavelength which may also be overcome by using an alternative line.
4. Spectrometer stray light intensity depends on the efficiency of the optical system used. The stray light effects (always flat-shaped) may be overcome by using adequate background correction.

4.3 Atomic absorption spectrometry

Introduced by Walsh in 1955 [22], atomic absorption spectrometry (AAS), has seen a more rapid growth than any other analytical technique since its commercial introduction in the sixties. Over the years, knowledge of the technique, optimization of optics and electronics, automation, and elaborate computer systems for handling large numbers of data have made this technique one of the best and most widely used analytical methods for the determination of major and minor elements in agricultural, environmental, biological, geological, industrial *etc.* samples. Together with ICP optical emission techniques, AAS seems to be replacing the traditional wet chemical methods and classical arc/spark emission devices intensively used in the past.

At the present time the analyst is confronted with an increasing demand for achieving a greater sensitivity, reliability and speed in analyzing complex samples. Modern technology has provided new reagents, procedures and instruments. In this section, an attempt has been made to provide the practising analyst or research scientist with a concise, convenient and critical guide through the vast literature of AAS, in a simplified form. Due to the greater complexity of furnace techniques, these are described in somewhat more detail than the already well-documented flame techniques.

4.3.1 Basic principles and instrumentation

The basic principles of atomic absorption spectrometry can be expressed by three simple statements: (i) all atoms can absorb light, (ii) the wavelength at which light is adsorbed is specific for a particular chemical element and (iii) the amount of light absorbed is proportional to the concentration of absorbing atoms.

An atomic absorption spectrometer is composed of:

1. A primary source to generate light at the wavelength which is characteristic of the analyte;
2. An atomization device to create a population of free analyte atoms;
3. An optical system to direct light from the primary source through the atom cloud and into the monochromator;
4. A monochromator to separate light at the analyte wavelength from all other light;
5. A light-sensitive detector and suitable electronics to measure and translate detector response into a useful analytical signal.

The principle of operating an atomic absorption spectrometer is that the primary source is used as the resonance line source. This primary source is usually a hollow cathode lamp where the cathode contains the element to be determined. The light beam consists of resonance radiation which is electronically or mechanically pulsed. When a sufficient voltage is applied across the electrodes, the filler gas inside the lamp is ionized and the ions are accelerated towards the cathode. As these ions hit the cathode, they cause the cathode material to sputter and form an atomic vapor in which atoms are present in an excited electronic state. In returning to the ground state, the lines characteristic of the analyte are emitted and passed through the atomization device. Other types of primary sources (EDL lamps or Superlamps with an additional boost-discharge electrode) can be used in some cases, principally for elements determined in the far UV wavelengths (As, Se *etc.*).

The ground state atoms which are produced in the atomizer (usually flame or furnace), and which predominate under the experimental conditions, absorb resonance radiation from the primary source, reducing the intensity of the incident beam. A monochromator isolates the resonance line and allows this radiation to fall on a detector. An electrical signal, whose magnitude depends on the light intensity, is produced. The electronic device is designed to respond selectively to the modulated radiation from the primary source, measure the light attenuation by the sample and convert these readings to the actual sample concentration.

Atomic absorption spectrometry is a comparative method. In practice, quantitative analysis is a matter of converting samples and calibrants into solutions, comparing the instrumental responses of calibrants and samples, and using these comparative responses to establish accurate concentration values for the element of interest. Basically, this can be carried out using simple equipment and simple procedures. Inevitably, however, there are aspects of the technique which are not quite as simple and straightforward as this brief introduction suggests. These aspects will be discussed more extensively in the following sections.

The use of automated sampling devices and automated recording of results is an effective means of increasing analytical productivity. Automatic sample exchangers usually take the form of a rotating or rectilinear table from which samples are successively presented to a capillary attached to the nebulizer. Similar sample exchangers are also used to present the samples to a graphite furnace. Similarly to spectrometers,

sample exchangers have benefited the development of microprocessor technology. Microprocessor- or computer-controlled spectrometers and sample exchangers/dispensers can be set up to interact in a very sophisticated way. The software of modern instrumentation provides extensive possibilities for routine analyses. Generally, a sequence of computer programs are stored on disk. These allow prerecording, storage and recall of analytical methods. The programs include a control of the possible baseline drift, specific algorithms for data manipulation and processing, such as calibration calculations and curve fitting, standard addition regression, averaging, standard deviation, quality control criteria *etc.* Details of the electrothermal program and both specific and background absorbance time profiles can also be displayed. This represents a very important improvement for method development and research of optimal parameters in furnace analyses.

4.3.2 Flame atomization

A successful atomic absorption determination depends on the generation of a supply of uncombined analyte atoms in the ground state and their exposure to light at their specific absorption wavelength. This process consists of taking a solution of the analyte and heating it to a temperature that is sufficient to dissociate the occurring compounds. Usually, the thermal energy required is supplied by a flame. The more sensitive but more problematic electrothermal (furnace) atomic absorption spectrometry is, however, now widely used for trace and ultra-trace analysis.

For the atomization, the sample is usually sprayed into the flame in the form of a solution by means of a pneumatic nebulizer. A flame is simple, inexpensive, easy to use, and provides a stable environment for atomic absorption. The complete process includes nebulization of the sample, the selection of mist droplets of the correct size and distribution, the mixing of the selected mist with the flame gases and its introduction into the burner which ensures the atomization. Due to the continuous and stable signal supplied by the flame AAS, the sensitivity of the method is given by the so-called "characteristic concentration", defined as the concentration of the analyte (in $\mu\text{g} \cdot \text{ml}^{-1}$) that produces a 1 % absorption signal (0.0044 absorbance) in the flame.

4.3.2.1 Nebulization

In a typical burner-nebulizer system, the sample is transferred into the nebulizer by the low pressure created around the end of the aspiration capillary by the flow of the carrier gas, and passed through the section. The liquid stream is transformed into a droplet spray by a carefully positioned obstacle (usually a spherical glass bead) and is ejected with the carrier gas into the spray chamber. During nebulization some liquids form a finer mist than others. Only about 10 % of the solution is converted into sufficiently fine droplets to be carried into the burner. The droplets with a diameter greater than about $5 \mu\text{m}$ fall out onto the sides of the chamber and flow to waste. The fuel gas is introduced into the chamber along with the carrier gas, and a mixture of fine sample mist, fuel, and carrier gas is transferred from the spray chamber to the burner. Differences in the uptake rates between samples and calibrants will clearly affect analytical accuracy, and nebulization must be identical for all samples and calibrants in a particular analysis. Since the amount of fine mist per unit volume reaching the flame affects the magnitude of the absorption signal, it is important to nebulize samples and calibrants of similar physical characteristics (solvent composition, surface tension, dissolved salts *etc.*).

4.3.2.2 *Flames*

The main function of the flame is to convert the analyte to the atomic state. Considering that the gases in the flame, and thus also the sample constituents, flow with a relatively high velocity, atomization processes should occur as quickly as possible. Measurement of the absorbance should be performed at a position in the flame at which either atomization is complete or equilibrium has been reached. In modern flame atomic absorption spectrometry (FAAS), air-acetylene and nitrous oxide acetylene mixtures are almost universally used for routine analyses.

The best known and most often used flame in atomic absorption analysis is the air-acetylene flame, generally used with a long path burner. The air-acetylene flame is convenient and safe for working at wavelengths above 200 nm, with minimum chemical interference. Its temperature is about 2300 °C. This flame can be used successfully for the elements that do not form refractory oxides and thus are easily atomized. Temperature is not always the main decomposition factor. Within each flame type the chemical nature of the flame (oxidizing, stoichiometric or reducing) will also have a profound effect on the decomposition behaviour of many elements.

Only in a few cases do noticeable ionization interferences occur (alkali metals), with a consequent change in the spectral response. The degree of ionization is different for each element, depending on the energy required to remove electrons. This energy can be supplied in various ways, but for atomic absorption the major source is the heat of the flame. The degree of ionization increases as the analyte concentration decreases; consequently, instead of the more normal calibration graph (nominally linear but in practice curving towards the concentration axis at high absorbance), the curvature tends towards the absorbance axis. This concerns only the ionization in pure solution of the particular element. The presence of any other element with an ionization potential close to or lower than the analyte will modify the extent of ionization significantly. In practice, the effective means to avoid interference due to ionization is to "buffer" the calibrants and samples with a high concentration of an easily ionized element. Provided that the concentration of this element is much greater than the analyte element, and its ionization potential lower, essentially complete suppression of the analyte ionization may be achieved. Due to their low ionization potential, sodium, potassium and cesium are the most commonly used ionization buffers.

Nevertheless, the temperature of the air-acetylene flame is insufficient to dissociate a substantial number of principally oxidic bonds or to prevent their formation in the flame. Certainly the most important development in the flames was the introduction of the nitrous oxide-acetylene by Willis in 1965 [23]. As a result of its low burning velocity, this hot flame (2900-3000 °C) offers a favourable chemical, thermal and optical environment for virtually all metals that give difficulties in the air-acetylene flame.

From the previous discussions it is evident that the analyst can maximize the population of analyte atoms in the flame by considering the adequate flame type, suitable flame stoichiometry and appropriate solution chemistry. However, before effective analytical measurements can be made it is necessary to ensure that light at the characteristic wavelength passes directly through the analyte atom population. Free analyte atoms are not distributed evenly within the flame envelope, and under given flame conditions there will be a particular zone which is more densely populated by the analyte atoms than other parts of the flame. The location of this zone within the flame is not identical for all analytes, and there is no single fixed position of the burner which would be suitable for all analytical situations. It is therefore necessary to adjust the burner position for

each separate analysis so that the maximum population zone coincides with the optical path. It is also important to remember that altering the fuel-to-oxidant ratio, or changing the total gas flow without altering the stoichiometry can modify the location of the maximum population zone within the flame. Consequently, it is often necessary to readjust the observation height in the flame until maximum absorbance is again obtained.

4.3.2.3 *Flame microsampling*

In this technique, a very small volume of the sample (typically 50-100 μl) is injected into a polypropylene or teflon funnel connected to the aspiration capillary of the nebulizer-burner system. The small volume of the sample reaches the flame and is atomized. The resulting analytical signal is a fast, transient absorbance peak, which is measured by the peak reading system (peak-height or peak-area mode). Direct injection of samples and calibrants is accomplished using a micropipette of the required volume. With recent instruments, flame microsampling can be performed more easily using the automatic sample dispenser. The major application for microsampling is the determination of relatively high concentrations, or in trace analysis where preconcentration techniques are required. In this case more elements can be determined from a given small volume than by conventional aspiration. Typically, up to ten elements can be determined from 1 ml of sample. Flame microsampling can also be used in many organic solvent applications and high-dissolved solid analyses where conventional aspiration may be troublesome.

4.4 Vapour generation techniques

Analytical requirements for some important elements are often such that the significant analytical level is below the detection limit of conventional flame methods. For some of these elements, special vapour generation techniques can be used to provide the required improvement in the measurable concentration.

4.4.1 *Hydride forming elements*

Antimony, arsenic, bismuth, lead, selenium, tellurium and tin can be determined by chemically reducing the element to gaseous hydride form and then dissociating the hydride in a heated quartz tube placed in the optical path of the spectrometer. Chemically, the procedure is simple. The reduction is performed by adding sodium borohydride pellets or solution to the appropriately acidified sample. The reagent concentrations are chosen to give quantitative evolution of elemental hydride vapour in a few seconds at room temperature. The hydride vapour is then swept by a continuous stream of nitrogen into a quartz tube heated in air-acetylene flame or electrically. The hydride compounds are atomized and the transient absorption signal is measured as a self-defined peak while the vapour passes through the quartz tube. This derivatization procedure is described in detail for speciation analysis elsewhere in this volume.

4.4.2 *Mercury*

4.4.2.1 *Determination*

Mercury determinations can be carried out using the described hydride generation system, but the formation of the vapour and the heating of the quartz tube are not required because of the volatility of mercury in the elemental state; hence, it can be directly measured with the cold vapour technique. In this case the atomic absorption

spectrometer is reduced to its absolute minimum: a mercury lamp, a quartz-windowed absorption cell and a specific photodetector at 253.7 nm. The mercury ions present in the acid solution after the mineralization step are therefore reduced to zero valent mercury in a reduction-aeration cell. The use of stannous chloride as reducing agent is preferred to sodium borohydride because a better precision and lower detection level can be achieved. The mercury vapour is then swept out through a drying tube into the quartz cell and the absorption signal is measured. This method was firstly described by Hatch and Ott [24].

For environmental studies, the determination of very small concentration of mercury (below the ng.g^{-1} level) due to the high toxicity of this metal and its compounds is required. Several preconcentration techniques have been proposed, based on absorption of elemental mercury in liquids or on solid phases. The preconcentration of mercury by amalgamation of a metal surface allows a reversible absorption/desorption; quartz sand coated with gold is a very efficient absorber due to its large active absorbing surface [25], the mercury is liberated by thermal desorption at approximately 800 °C and introduced into the atomic absorption spectrometer. Quartz tubes filled with gold coated sand can be used for the collection and determination of gaseous mercury in air while mercury compounds associated with particulate matter are retained on a quartz prefilter.

4.4.2.2 Sources of errors

Spectral interferences associated with the determination of mercury by cold vapour atomic spectroscopic methods, mostly due to the presence of sulphur dioxide, ammonia or aromatic compounds in the gas stream, can be eliminated by using a gold film mercury detector, based on a linear change in resistivity of a gold film as a function of the degree of amalgamation [26].

Due to the high volatility of mercury and some of its compounds, and its property to adsorb on most surfaces, many systematic errors can occur at the ng.g^{-1} level; they are mostly associated with the treatment, storage and mineralization of the samples and are well described by Kaiser *et al.* [27]. Contamination by containers and glassware is often a cause of high background values.

4.5 Electrothermal atomization

The first electrothermal atomizers for AAS were developed in the early seventies and allowed a considerable improvement in sensitivity. However, this technique suffered from numerous spectral and non spectral interferences which rendered it difficult to handle. A major improvement was made with the introduction by L'vov [28] of a platform into the atomizer to reach near isothermal atomization and the use of efficient chemical modifiers to increase the pyrolysis temperature and thus attain a more specific volatilization of the analyte during the atomization step. These two points made it possible to reduce or totally overcome the influence of most analyzed matrices.

4.5.1 General considerations

Electrothermal atomizers have provided a considerable improvement in sensitivity for the majority of the elements determined by atomic absorption. This is due essentially to the introduction of the entire sample into the atomizer, thereby overcoming the inefficiency of nebulization systems and avoiding the large dilution of the atomic population in the flame gases.

Flame and furnace atomizers provide the same end-product: a supply of free analyte atoms for exposure to light at a characteristic wavelength. However, there are major differences in the mechanisms of molecular dissociation and in the overall efficiency of the atom production process in both systems. In flame technology, the chemical composition of the flame, rather than pure temperature, is generally more important in maximizing the free atom fraction. The conversion efficiency is low: typically only about 10 % of the aspirated sample is converted into the free atom population for measurement in the optical path. With electrothermal atomizers, molecular dissociation is governed by the final temperature used, by the rate at which this temperature is attained, and by the reducing environment of the hot graphite. Conversion efficiency is typically high since all of the available sample is used to produce the atom population within the optical path. Thus for a given analyte concentration, the atom population in the furnace atomizer will be considerably more dense than in the flame. Consequently the measured absorbance will be considerably higher. In practical analytical terms, measurements within the useful absorbance range can be obtained at concentrations considerably lower than with flame methods.

4.5.2 Atomizer

The graphite tube is mounted in the atomizer head between two graphite electrodes located in the water-cooled metal block. A toggle mechanism locks the graphite tube in place and allows the furnace assembly to be opened for replacement of the tube. The contact area between the electrodes and the tube is relatively small. The tube is thus resistively heated by the passage of a high current at low voltage. The heating (achieved temperatures up to 3000 °C and ramp rates up to 2000 °C.s⁻¹) of the tube is monitored by an adequate temperature control device. Inert gas (usually argon) flows through the electrodes to protect the inner and outer surfaces of the graphite tube from rapid oxidation by atmospheric oxygen. The graphite tubes are coated with pyrolytic graphite which is, compared to normal graphite, impermeable to solutions and gases. Pyrolytic graphite is also more resistant to oxidation than normal graphite, and is inert.

4.5.3 Electrothermal program

For routine analyses, a small volume of sample (typically between 3 and 50 µl) is introduced into the atomizer through the port at the top of the tube, generally by means of an automatic sample dispenser. The graphite tube is then heated in accordance with a pre-determined electrothermal program. Although there are different approaches in the detailed design and construction of furnace atomizers, they all perform according to the same fundamental process: generating a population of free analyte atoms so that atomic absorption can be measured. In its simplest form, the electrothermal program is achieved in three stages:

1. The drying step: the solvent is evaporated from the sample at a temperature near the boiling point (± 100 °C and about 2 s per µl for aqueous solutions). The evaporation must be gentle in order to avoid losses by sputtering. When the drying stage is complete, the residue in the atomizer tube will consist of a thin crust of material, containing the analyte element together with all the solid components of the sample matrix.

2. The ashing (charring, pyrolysis) step which removes the organic or inorganic matrix. This step represents the most important stage of the electrothermal program. The matrix is thermally decomposed at an intermediate temperature (300-1500 °C as a function of the analyte). The objective is to remove the maximum of the matrix while keeping the analyte entirely within the atomizer in a stable form so that atomization can proceed with a minimum interference from the matrix components. During the ashing step, an alternative gas (generally air or oxygen) can be used to achieve a better matrix decomposition. When the ashing step is complete, the residue should consist of the analyte element in its appropriate molecular form, as a minimum of the matrix inorganic salts which are thermally stable at the ashing temperatures used.
3. The atomization step in which the dissociation of the analyte molecular species at a high temperature (up to 3000 °C) occurs, and free analyte atoms are generated within a confined zone coincident with the spectrometer's optical path. The flow of the sheath gas is usually interrupted during the atomization in order to extend the residence time of atoms in the optical path and consequently to enhance the sensitivity of the atomic absorption measurement, performed during this step.

While it is convenient to consider the electrothermal program as a three-stage programming, it is actually divided into a number of small steps. Each step can be separately programmed for duration, rate of temperature rise (ramp parameters) and temperature at the end of the step (hold parameters). Before performing a real analysis, the ashing and atomization temperatures for an element in a particular matrix must be defined using preliminary programming. Double curves are established, in which the absorbance signal is plotted versus the applied temperature. In the first curve the height of the signal at the fixed atomization temperature (found in the operating conditions supplied by the manufacturer) is plotted versus the ashing temperature as the variable. This curve shows the temperature to which a sample can be thermally pretreated without loss of analyte element, the "optimum ashing temperature". In the second curve, this optimum ashing temperature is fixed and the absorbance signal is plotted versus the now variable temperature of atomization. The temperature at which the maximum atom cloud is attained (maximum absorbance signal) is called the "optimum atomization temperature".

In electrothermal AAS, the absorption signal produced during the atomization stage is a transient, well-defined peak. The height and area of the transient absorbance peak from the ETAAS are related to the amount of analyte present in the sample. By analogy to flame AAS, the sensitivity of furnace analysis is called "characteristic mass", m_0 , and is defined as the amount of the analyte in mass units (pg) that produces 0.0044 absorbance (for peak-height measurements) or 0.0044 absorbance.second (A.s), (for peak-area measurements). When the slope of the working curve is known (in A/pg), the characteristic mass can be calculated as $m_0 = 0.0044/\text{slope}$.

4.5.4 Interferences

The presence of the sample matrix can lead to interferences. The matrix effect, for example, is a composite interference due to all the concomitants in the sample. The other phenomena (influence of the flame, graphite, sheath gas *etc.*) are not considered as interferences since they are not due to the sample properties.

4.5.4.1 *Spectral interferences*

Spectral interferences mainly arise from a scattering of the source radiation on non-volatilized particles from concomitants or from absorption of the source emission line by overlapping atomic lines (in AAS this probability is very low and the problem is well documented) or from the molecular bands of the concomitants.

Problems arising from background absorption (light scattering and molecular absorption) are more frequent with electrothermal atomizers than with flames. In chemical flames, background absorption is rarely significant at wavelengths higher than about 240 nm, and the background signal seldom exceeds about 0.05 absorbance. In furnace analysis, background absorbance can be significant at wavelengths up to about 500 nm, and the background signals may exceed 2 absorbance units.

Light scattering, which produces a broad band absorption, is caused by the condensation of the sample matrix, after vaporization, by forming a smoke or mist; this event occurs when a huge amount of volatilized matrix reaches the cooler regions at the open ends of the graphite tube. Molecular absorption is caused by the broad absorption bands of molecular species present in the atomizer, particularly alkali-metal and alkaline-earth halides. In practice, all these phenomena are observed when analyzing samples with high dissolved salts, mainly halides in sea water, urine *etc.* Sharp absorption lines, called "structured background" can be superimposed on the broad absorption bands, due to electronic spectra of molecules.

To counter the background phenomena, there are three complementary counter measures available to the analyst: simultaneous background correction, appropriate temperature programming, and the use of chemical modifiers.

An essential part of an atomic absorption system for many applications, particularly for electrothermal atomization methods, is a device for correcting the atomic absorption signal for non-specific molecular absorption or light scattering effects. Both of these attenuate the light beam, giving an apparently increased absorption signal. A simultaneous background correction device is very convenient in flame work, but absolutely essential in analysis using a furnace. A well-established technique for correction is to measure, in rapid sequence, the radiation attenuation of a primary source which measures the total absorption (the sum of analyte atomic absorption and background absorption), and of a continuum source (background absorption only). A further technique for exact background correction introduced in recent years makes use of the Zeeman effect.

Background correction with a continuum source. Systems for background correction using a continuum source are based on the work of Koirtzmann and Pickett [29]. In these devices the radiation from the hollow cathode lamp is passed alternatively (mechanical or electronic chopping, pulsing, modulating) with the radiation of a continuum source through the graphite furnace. The optical configuration is such that radiation from both the hollow cathode lamp and the continuum lamp coincide precisely along the optical path through the observation zone. After passing the monochromator, both radiation beams fall on the same detector and an electronic measuring system forms the ratio from both radiant intensities. The background signal is subtracted electronically from the total absorbance signal and the analytical result is thus corrected for background interference. Most often, the background corrector consists of a deuterium arc emitting a continuum signal (190-360 nm). The deuterium and cathodic radiations are focused

in the centre of the furnace with equivalent energies. During the reading, the non-specific absorptions attenuate the two beams in the same way, while the analyte element absorbs only the cathodic beam because of its narrow line width.

It is worthwhile considering at this point how to ensure that the background correction operates with the best possible efficiency. This requires attention to the optical alignment and the chopping frequency. In order to ensure that correction is applied only to the volume within the tube where the atomic absorption is measured, it is essential that the continuum beam be accurately aligned with the atomic beam within the atomizer.

The rate at which a cycle of pulses occurs (chopping frequency) is of great importance when the corrector is working with an electrothermal atomizer. As already noted, during the atomization step the atomic population increases quickly to a maximum, then falls more slowly. The background absorption behaves in a similar way, though not identically. The accuracy of correction therefore depends on the time interval (spectrometer time constant) between successive pulses relative to the rate of change of both background and total absorption.

The background correction with a continuum source has disadvantages and limitations. In general, it should be attempted to keep the background attenuation below 0.5 absorbance; frequently, higher background values may be incompletely corrected, especially if fast, dynamic signals are generated by the graphite furnace. For samples of unknown composition, it is important to have information of the nature, magnitude and rate of apparition of background signals. For this purpose, most modern atomic absorption spectrometers permit the detailed observation, on a screen display, of both specific and background absorbance signals generated during the atomization step. The spectral continuity of the background signal with the bandpass of the monochromator is an essential condition for a good correction. This is assured for light scattering and dissociation continua. Background correctors using a continuum source are incapable of dealing with background attenuation of electronic excitation spectra, composed of many narrow lines. For this type of spectrum, the actual background correction depends on the degree of overlap between the analyte spectral line and the individual molecular rotational or vibrational lines [30,31]. If a continuum background corrector is used in these conditions, it determines the mean absorbance over the observed spectral bandpass, and this mean absorbance may be higher or lower than the actual attenuation at the analyte wavelength. This results in an over- or an undercompensation when the background readings are subtracted, and thus an invalid analytical measure. From the practical view-point, the problems due to the structured background concern fortunately only the determinations of selenium and arsenic in magnesium and calcium phosphate matrices (plant and animal tissues, soils, blood, urine...). For this applications, the Zeeman background correction is thus strongly recommended.

Zeeman effect background correction. A fundamentally different approach to background correction involves the Zeeman effect which occurs when an atomic vapour which is absorbing or emitting resonance radiation is subjected to a magnetic field of several kilogauss. This procedure splits the spectrum lines into a number of components. In the simplest cases, the main resonance line is replaced by a π component situated at the original wavelength together with two σ lines, displaced by equal wavelength intervals at both sides of the original line. Splitting of the spectral lines into three components is designated the *normal Zeeman effect* and concerns the main resonance lines of Group IIA and IIB elements. All other lines exhibit an *anomalous Zeeman effect* and split into more than three components.

These effects can be used in background correction by applying the magnetic field (permanent or alternating) either to the source of the resonance radiation (*direct Zeeman effect*, compatible with conventional hollow cathode lamps) or to the atomized sample (*inverse Zeeman effect*, the most popular). Besides the Zeeman splitting of the resonance lines, the radiation is also polarized. The polarization varies according to the direction of observation and the resulting configurations are called the *transverse Zeeman effect* (magnetic field perpendicular to the radiation beam) or the *longitudinal Zeeman effect* (magnetic field parallel to the radiation beam). In most commercial systems, the light from the source is polarized alternatively, by means of a rotating polarizer, in planes perpendicular and parallel to the magnetic field applied to the atomizer, before it actually passes through the atomizer. With the Zeeman effect, only light polarized in a parallel plane is absorbed by the π components (atomic absorption and background attenuation). Light polarized in a perpendicular plane does not undergo any atomic absorption and only submits an attenuation by the background. These components are detected in the usual way. For practical applications it was shown that the optimum Zeeman system has a magnet located at the atomizer (inverse Zeeman effect), operating with an alternating field at a flux density of about 1 Tesla. For practical reasons regarding the instrumentation, the transverse Zeeman effect was first selected. This configuration offers an optimum double-beam system, high accuracy background correction, linearity and sensitivity similar to conventional AAS and detection limits better than can be achieved with a deuterium arc background correction. Such a system permits a correction of background signals up to 2 units of absorbance. The background measurement is performed exactly at the analyte wavelength: the Zeeman background correction systems can thus deal with structured background, uncorrectable with conventional deuterium devices. Recently, a longitudinal Zeeman system was also introduced. This system theoretically provides a better performance than the transverse configuration, due to a higher energy throughput by omitting the polarizer.

4.5.4.3 Non-spectral interferences

Non-spectral interferences are the major problem when using electrothermal atomizers. They are usually classified according to the place, stage or process responsible for their occurrence, *e.g.* condensed-phase interferences concern all processes occurring from the formation of compounds and reactions during drying and ashing steps to the complete volatilization of the analyte from the graphite tube. Vapour-phase interferences occur when the analyte is not completely dissociated into atoms in the ground state.

Interferences in the condensed phase include all influences on the analyte up to the moment when it volatilizes and leaves the hot graphite surface. They are due to *e.g.* losses of the analyte during the ashing step, to the formation of carbides or intercalation compounds, to the occlusion of the analyte by the matrix, and similar processes that may result in changes of the analyte volatilization rate or its incomplete atomization. Furthermore, one of the main causes of vapour phase interferences in electrothermal atomizers is the formation of monohalides, principally in the presence of chlorides in the sample (sea water, urine) or in the acid mixture used for the mineralization of the sample (HCl). Most analytes form stable molecules with the halides at the decomposition stage. These molecules are volatilized during the atomization step but they are not dissociated into free atoms. The molecular absorption produced by the undissociated molecules is, evidently, corrected as a non-specific absorption, and this part of the analyte is lost for atomic absorption.

In comparison with an absorbance signal measured in a simple aqueous medium, a non-spectral interference usually provides a change in the absorbance-time profile obtained in the sample (lowering, enhancement, enlargement *etc.*). Consequently, the slopes of the working curves established in both simple and complex media differ, and the direct calibration method against aqueous calibrants is not valid. In these cases, the following countermeasures can be of use.

Standard addition method. In general, non-spectral interferences can be eliminated by making calibrants as similar as possible to the sample composition. In the ideal case, a calibrant would contain not only the same solvent, but also the same concomitants. Fortunately, interferences are rarely so pronounced that calibrants should match sample solutions exactly. When the sample composition changes markedly or more complex matrix effects are encountered, then the standard additions calibration method is recommended. As a general rule, this procedure should provide a substantially linear calibration since accurate regression cannot be obtained from non-linear calibration points. It is also essential to establish an accurate baseline from the appropriate reagent blank. In several applications of the standard additions method, each sample has to be analyzed individually, *e.g.* in the case of samples of similar origin but with very different matrices. The most variable matrix from one sample to another can be found with urine samples, for which trace element determination thus becomes particularly difficult. There are some situations in which a batch of samples can be analyzed against the one set of standard additions, but this is only valid when all samples in the batch are chemically and physically similar (*e.g.* similar matrix from one sample to another: seawater, blood). Using the standard additions method, it should be possible to compensate all non-spectral interferences, provided that they are independent of the analyte concentration. With modern instruments the standard additions method can be performed automatically but it is time-consuming and, hence, less attractive for routine purposes.

Atomization off the platform. In the original furnace concept, the graphite tube is heated to a high temperature and the sample is vaporized from the tube wall. This quickly converts the sample into an atomic vapour that absorbs light from the primary source. With the Massman-type furnaces that are currently used, it is not so simple. If a sample is placed on the wall of the graphite tube, the various sample concomitants will vaporize as the wall of the tube heats up, and into an environment which is much cooler than the wall of the tube directly heated. In these conditions, recondensation of the analyte (and sample concomitants) may occur, resulting in a depression of the absorbance signal. However, the temperature and rate at which the volatilization occurs depends on the compound in which the analyte is present. With such differences in volatility, the residence time of analyte atoms in the atomizer will be different as volatilization occurs into an atmosphere that is rapidly increasing in temperature. This is in contradiction with the assumption of the initial L'vov theory that all atoms, regardless of their form, will be volatilized into an atmosphere that is at the same constant temperature.

As a solution to this problem, L'vov suggested that the heating of the sample be decoupled as much as possible from the heating of the graphite tube. This was achieved by placing a small, solid pyrolytic graphite platform on the bottom of the graphite tube [28], the sample aliquot being then deposited on this platform. The temperature of the platform, heated primarily by radiation, lags behind that of the tube wall. Hence, vaporization is delayed until the atmosphere reaches a high and nearly constant temperature. The analyte is volatilized from the platform into a gaseous environment which is at higher temperature than the atomization support. This leads to an efficient

decomposition of molecules, minimizes recondensation phenomena and, consequently, drastically reduces vapour-phase interferences. With respect to volatilization from the tube wall, the volatilization from the platform is delayed, and each atom, regardless of the temperature at which it is volatilized, will have the same residence time and contribute equally to the absorption process.

From a practical point of view, the use of the platforms also extends the useful lifetime of the atomization support. While graphite tubes are coated with only a thin layer of pyrolytic graphite (typically about 50 μm thickness), platforms are made of solid pyrolytic graphite, more resistant to degradation by corrosive agents (acids, oxygen *etc.*) or samples (seawater, urine *etc.*).

Chemical modifiers. Chemical modification techniques, first used by Ediger *et al.* [32], are now widely used in electrothermal atomic absorption spectrometry. They are generally employed in the determination of various volatile and mid-volatile elements, generally atomized off the platform. For the determination of refractory elements, atomized from the tube wall, chemical modification is used more rarely.

A chemical reagent (modifier) is added in large excess directly to the calibrants and samples, or to the atomizer before the addition of the analyzed solutions, to convert the analyte into a phase of higher thermostability (*analyte modifier*), to increase the volatility of the concomitants (*matrix modifier*), or for both purposes (*analyte/matrix modifier*). In the analysis of complex and difficult samples the use of *complex (or composite)* rather than *single-component (individual)* chemical modifiers is often preferred. Their various constituents have different roles during the thermal pretreatment of the sample (for example the analyte thermal stabilization and the real modification of the matrix). The action of the usual modifiers is given in Table 1.

Analyte modifiers. In most cases, the modifier retains the analyte up to higher temperatures. This, on the one hand, permits matrix components to be removed more easily and more effectively prior to the atomization of the analyte. On the other hand, thermal stabilization of the analyte to higher temperatures results in its volatilization in a hotter vapour phase which is closer to thermal equilibrium. This fact minimizes substantially the risk of vapour-phase interferences.

The best example of this kind of modifier is represented by palladium. Palladium is a very effective chemical modifier and can be used to stabilize many elements to several hundred degrees (300-1000 $^{\circ}\text{C}$) higher than the temperatures possibly obtained with current methods [33]. The greatest temperature shifts are achieved for the following elements: As, Se, Bi, P, Pb, Sb and Tl. The increase of the appearance temperature is attributed to the formation, with palladium, of more refractory intermetallic species or alloys. Other metals of Group VIII (in particular Ni, Ir and Pt) can be also very efficient modifiers in some cases, but the "universality" of palladium is clearly demonstrated.

Table 1 - Action of some chemical modifiers

Type of matrix or analyte	Modifiers, action
Matrix with alkali-metal and alkaline-earth halides	Formation of more volatile compounds NH ₄ nitrate, phosphates ascorbic or oxalic acids
	Formation of more volatile compounds hydrofluoric acid
Ba, Mo, Ti, V, U, Zr	
Ag, Cd, Pb As, Au, Bi, Sb, Se, Te Tl P Hg Mn	Formation of more refractory compounds NH ₄ phosphates, Pd nitrate, Ni, Cu, Pd, Ag, Mo, Fe nitrates, Ir (hexachloroiridate) H ₂ SO ₄ , Pd nitrate Pd, La nitrates Pd nitrate Pt (hexachloroplatinic acid), Pd nitrate
Al, Be, Cd, Co, Cr Fe, Mn, Ni, Pb, Sn	Incorporation into a salt or oxide alkali-metal or alkaline-earth nitrates, usually Mg(NO ₃) ₂

An increase in the volatility of the analyte by means of an analyte modifier can also be used. In this case the modifier acts as a "*volatilizer*", facilitating an atomization at low temperature. These applications, generally using carboxylic acids as modifiers, facilitate the temporal separation of the analyte and background signals. They are employed for the determination of the most volatile elements (*e.g.* Cd, Zn) in the matrices which generate huge background signals (seawater, urine). Similarly, by using adequate modifiers (generally fluoride-based), the atomization temperature of several refractory elements can be lowered (*e.g.* Mo, V). Decreasing the atomization temperature has a beneficial effect on the graphite tube lifetime and long-term reproducibility of the determinations of the refractory elements.

Matrix modifiers. The role of a matrix modifier is to increase the volatility of interfering concomitants. The matrix can be extracted during the pretreatment stage with a better efficiency. In this case the atomization of the analyte is less disturbed by the possible non-spectral interferences and high level background signals. Their use is not frequent; in practice only ammonium nitrate or nitric acid are largely employed to volatilize chlorides from seawater or urine samples prior to atomization. For a more efficient removal of organic matter from the sample during ashing (*e.g.* for blood trace-metal analysis), the use of oxygen as an alternative gas can be considered as a matrix modifier. In addition, "ashing aids" have beneficial effects on the organic matrix modifications and simplifications during the thermal decomposition. Magnesium nitrate or nitric acid (generally in combination with another modifier) are mostly used to favour the oxidation processes and to facilitate a more efficient removal of the matrix prior to atomization.

Analyte/matrix modifiers. The same chemical modifier acts also on the matrix components during the ashing stage, and modifies their influence during the atomization process of the analyte. For example, in the determination of selenium in a phosphate matrix using a deuterium background correction device, the uncorrected absorbance signal, due to structured background from generated phosphorus species, is shifted to higher temperature values in the presence of palladium (or nickel, iridium *etc.*) added as a modifier. The analyte absorbance signal is, consequently, not longer superimposed on the background signal and hence, uncorrectable with a conventional deuterium arc device. In these conditions, the selenium absorbance signal can be measured away from the influence of the structured background [34]. In this example, palladium (or nickel) first acts as a thermal stabilizer of selenium, but also modifies the volatilization behaviour of the phosphate matrix.

Complex or composite modifiers. The beneficial properties of palladium as a matrix modifier have already been widely demonstrated. However, its use as a single modifier cannot solve all interference problems encountered in furnace analysis of difficult matrices. The efficiency of palladium may still be reinforced in some instances by its combination with one or more other agents. For example, the mixed modifiers composed of palladium and of ammonium or magnesium nitrates were suggested by Welz and co-workers [35,36] for the efficient determination of a number of trace elements in several environmental matrices. Similarly, Beach and Shrader [37] employed a reduced-palladium modifier. The addition of a reducing agent (ascorbic acid, hydroxylamine, hydrochloride or hydrogen added to the sheat gas) ensured a more consistent performance.

The way is now open to elaborate complex modifiers. The easiest way, in our opinion, would be to use matrix interferents themselves to complete the action of an analyte modifier in order to simulate, as far as possible, the same analytical conditions encountered in both simple and complex media (calibrants and samples). Some of the main sample matrix elements act as interfering agents. In the presence of a basic analyte modifier (*e.g.* palladium), it is assumed that by adding these interfering elements in large excess to the samples and calibrants, the interferences produced by the samples themselves will be negligible. This assumption appeared to be valid for numerous types of furnace analyses of various environmental samples [38]. The following example describes the step-by-step procedure carried out to ensure an interference-free determination of lead in various environmental samples. In a simple aqueous medium, the ashing step for lead should not exceed 500 °C as, above this temperature, losses of the analyte will occur. When analyzing a complex sample under these conditions, the matrix is generally not sufficiently removed during the ashing stage, which results in a disturbed atomization of lead. With the addition of palladium, the charring temperature may be elevated to up to about 1200 °C, a temperature sufficiently high to remove an important part of the matrix. However, in the case of the analysis of simple aqueous calibrant, the lead atomization rate (peak-height) decreases (the palladium only acts as an interfering element in this case), whereas, in the analysis of environmental matrices (plant and animal tissues) the lead atomization process is largely accelerated (enhanced peak-height signal). This clearly shows that, in the presence of palladium, one or more matrix components contribute to this enhancement which is not observed in the simple aqueous medium. Additional experiments have shown that the different behaviour of lead during the atomization is due to the presence of phosphorus and magnesium, two main matrix elements of most environmental matrices; a mixed modifier composed of

palladium and of excess ammonium phosphate and magnesium nitrate may therefore be successfully used: palladium ensures the thermal stabilization of lead, phosphates accelerate its atomization process (by enhancing its peak-height signal), and magnesium nitrate acts as an ashing aid and allows normalization of the differences in the absorbance peak-shapes observed for the various matrices. Consequently, with such a mixed modifier, charring may be performed at 1100 °C, and the lead absorbance-time profiles for all the matrices studied, including simple aqueous medium, have similar shapes. This is verified by a similarity in the slopes of standard addition curves established in the different matrices, indicating the absence of non-spectral interferences. In this case, a direct calibration against aqueous calibrants is valid for matrices as different as soils, sediments, suspended matter samples, animal and plant tissues or sea water [39,40]. This example permits the evaluation of the great potential of chemical modifiers, and shows the probable fields of future research into the analytical applications of electrothermal atomic absorption spectrometry.

Another example is given by the determination of cadmium in seawater. Cadmium is one of the elements with a very high sensitivity (≈ 0.4 pg for 0.044 absorbance). In a simple aqueous medium, the pyrolysis temperature should not exceed 380 °C. This temperature can be increased in the presence of nitric acid [38] or other compounds as phosphates [41] but losses of cadmium can be expected at 600-700 °C. In saline samples, the cadmium and salt matrix are volatilized at the same temperature: both the analyte and background absorbance signals hence appear simultaneously which leads to difficulties in the cadmium determination. In seawater samples these background signals are too high to be satisfactorily controlled by deuterium or Zeeman correction devices. The use of platinum metals-based modifiers offers a possibility of substantial increase of pyrolysis temperature for several elements, *e.g.* Pb, Mn, Tl, As, Se, Cu, Te... [38]. Unfortunately, the determination of cadmium cannot benefit from this advantage. With palladium, a pyrolysis temperature higher than 700-800 °C cannot be used without provoking losses of cadmium. Such a temperature is generally sufficient for the analysis of matrices such as water, plant and animal tissues, soils and sediments, but for seawater analysis it is not enough to remove a significant part of the matrix during the pyrolysis step as uncorrectable background levels will be generated during the atomization step. Cadmium atomization at relatively low temperature, before the appearance of high background levels, requires a suitable temperature program: firstly the unnecessary charring step, which is in any case conducted at a temperature too low to remove the seawater matrix, is skipped. The drying of the sample is then directly followed by the atomization step. Possible losses of cadmium due to inadequate charring temperatures are thus totally avoided; secondly, cadmium is atomized at a temperature as low as possible to avoid the huge volatilization of the matrix and hence high background levels. The best results are obtained at 1400 °C, a temperature at which the atomization rate of cadmium is sufficient and the background signal is satisfactorily controlled by the correction device. The analyte signal is not directly subject to interference by the increasing background signal (as observed at a temperature up to 1500 °C) and the integration time is limited. The salts remaining in the atomizer after this low temperature atomization step are removed during an additional cleaning step conducted at 2400 °C. The situation is still improved by using an argon-hydrogen mixture as the sheath gas. In this case, the cadmium absorbance signal is entirely out of the background signal influence, and the limitation of the integration time is not necessary anymore [42].

4.5.5 Role of the graphite

Since the commercialization, in the early seventies, of graphite furnace atomizers, concern has arisen about the variability of the surface properties of graphite tubes and their progressive degradation with increasing numbers of firing. Although the use of spectrographic graphite has specific advantages, such as a mechanical resistance increasing with temperature up to 2500 °C and good reduction properties, several drawbacks exist. Firstly, graphite can interact with some elements to produce stable carbides and/or interlamellar compounds. Both of these phenomena may hamper the volatilization of analyte and change its original atomization process. Secondly, graphite is porous and the atomizer wall is therefore subject to infiltration by solutions. In addition, vapour can diffuse at high temperature through the atomizer wall. Coating of the tube wall with pyrolytic graphite, which is non-porous and highly impermeable to gases at the temperatures generally applied, has greatly reduced these problems [43,44]. However, the thin pyrolytic graphite layer itself becomes increasingly porous with tube aging as a result of the influence of strongly acidic solutions, corrosive samples and high atomization temperatures. Other coatings and impregnation materials have been studied in order to improve the atomizer surface properties. Metals with an elevated melting point and forming stable interstitial carbides with graphite (Ta, Mo, Nb and W) have often been used for this purpose [45,51]. However, improvements obtained by these treatments are too case-specific to be universally applicable. Thirdly, the quality of the pyrolytic coating may be highly variable and is a function of the number of active sites per unit area. This number depends on the initial number of nucleation centres available on the graphite surface during the coating process [52]. Oxygen provided by the injected sample solution will bind to carbon preferentially on these active sites. During atomization, this carbon is released in the vapour phase as CO gas, increasing the active site oxygen-binding capacity. Also, analyte and matrix elements will compete for these active sites. Such processes are variable during the tube lifetime and can favour or inhibit certain reactions [53]. They can also modify the initial process of analyte atomization. In practice, it is extremely difficult to distinguish between the different processes involved and to identify the ones which are controlled mainly by active site availability [54]. The situation described concerns mainly the determination of refractory elements. The determination of more volatile elements is less affected by these problems.

4.6 Atomic absorption spectrometry / conclusions

Flame atomic absorption spectrometry is now very well established, relatively inexpensive and easy to use: it requires little operator experience. The interferences are rare, well known and easily controllable. Only the refractory elements (V, Mo, Ta, W *etc.*) are not completely dissociated in the flame and are therefore not easily determined. Also elements that have their analytical lines at low wavelengths (As, Se, Te *etc.*) exhibit a sensitivity that is insufficient for the usual analytical problems. If the lowest detection limits that can be obtained with current spectroscopic methods are required, electro-thermal atomization is the technique of choice. On a relative basis, the detection limits obtained with a graphite furnace are 10-100 times better than with a flame. In the recent past, however, numerous interferences were reported and the use of this technique in routine work was practically impossible in many cases. These problems are now largely controlled using a combination of platforms, chemical modifiers, efficient background correction devices and modern spectrometric instrumentation. However, furnace

determinations remain slow - typically several minutes per element and sample. Also the analytical range is not very large, about 1-3 orders of magnitude. Thus, the electrothermal atomization must be employed only when the flame provides insufficient detection limits.

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5.

Validation of Neutron Activation Analysis Techniques

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Neutron activation analysis in its purely instrumental form (INAA) is conceptually an extremely simple method of analysis, involving only two completely separate processes, excitation and measurement. The excitation of the sample by exposure to neutrons, as well as the subsequent measurement of the induced activity by a suitable detector, are both based on well-known physical laws, and the content of an element in a sample may be directly compared with an accurately known quantity of the same element.

Unlike other methods of analysis, excitation and measurement are separated in time, so that a *radiochemical separation* (RNAA) can be carried out between the exposure to neutrons and the measurement of activity. In this way the activity of the determinand element can be isolated from a dominant or interfering activity from other elements, and RNAA therefore yields results with improved precision and accuracy.

In addition, unlike conventional chemical separations a radiochemical separation does not give rise to any blank value from added reagents, simply because a content of the determinand element does not contribute to the activity measured. This makes possible the intentional addition after irradiation of a known amount of carrier of the determinand element, just prior to the radiochemical separation. Such carrier addition not only reduces the risk of losing the determinand element by adsorption or other similar processes during the separation, but makes it possible to determine the actual recovery of added carrier after the final measurement of activity has been made. Such a determination of the *chemical yield* is used to correct the analytical result for losses associated with the separation; in a *comprehensive radiochemical neutron activation analysis* [1] the chemical yield is determined for each individual sample analyzed.

When a pure element or a well-defined stoichiometric compound is used as a comparator standard, neutron activation analysis is directly traceable to the fundamental units of the SI-system. The absence of a reagent blank and the accurate correction for losses of determinand eliminate significant systematic errors; if all other sources of variability can be brought in statistical control, NAA qualifies as a *Definitive Method* [1]. The characteristic properties of neutron activation analysis make it one of the most important methods for the certification of trace element concentrations in environmental reference materials. When each step of the analysis is carried out with meticulous care and insight, complete statistical control can be achieved in such certification analyses.

Lack of agreement between observed and expected variability among NAA results from different laboratories has also been observed in BCR certification projects; thus, even selected laboratories may fail to identify all significant sources of variability.

By systematic validation of the analytical techniques in each laboratory it is possible to eliminate bias and account for all significant sources of variability [2]. Such validation may be carried out using quality assurance methods based on the Analysis of Precision [3]; a validated method in statistical control yields unbiased results with an accurately known uncertainty.

5.1 Critical review

Regardless of the excellence of the analytical method, analytical results without an indication of their uncertainty are useless - they may even be dangerous, because they can be misused! A meaningful statement of uncertainty must include all potential sources of variability that could affect the analytical result, not only the variability observed by repeating the final measurements.

Even in certification analyses, where each analysis is carried out completely independently, all potential sources of variability are not always accounted for. In Figure 1 results are presented for Mn in a coal reference material CRM 182 as a mean and standard deviation of 5 replicates from 14 laboratories using 4 different methods [4]. It is easy to see that results from all 4 analytical methods are affected by unaccounted factors. Such unaccounted factors usually give rise to biased results, and the results should be disregarded until the unknown source of variability has been identified and corrected for.

5.1.1 The BIPM Philosophy

The proper way to evaluate and express uncertainties in neutron activation analysis and other methods that can be brought in statistical control [5] is to adopt the approach recommended by BIPM [6] and described by ISO [7]:

Based on an accurate description of the particular analysis carried out, the analytical method is divided into a number of independent steps 1...n. Each step is assigned an uncertainty in the form of a standard deviation, σ_i , either by applying statistical methods (**category A**) or by other means (**category B**). This itemization is referred to as an uncertainty budget, because it shows clearly which steps are most critical for the uncertainty of the analytical result.

The overall uncertainty $\hat{\sigma}$, or **combined uncertainty**, is now obtained by combining the variances from both categories

$$\hat{\sigma}^2 = \sigma_1^2 + \sigma_2^2 + \sigma_3^2 + \dots + \sigma_n^2 \quad (1)$$

according to the laws of propagation of errors.

Excess variability

The standard deviation $\hat{\sigma}$ calculated according to Eq. (1) is often referred to as the *a priori* precision [8], because it can be determined before any actual analyses are carried out.

After the completion of a number of n replicate analyses the *a posteriori* standard deviations are calculated from the variability of the actual results y_i

$$s^2 = \sum_{i=1}^n (y_i - \bar{y})^2 / (n-1) \quad (2)$$

In many cases it is found that the variability of actual results is larger than expected from the *a priori* precision, and considerable effort may have to be spent on identifying the source of this excess variability.

In neutron activation analysis and other methods involving counting procedures, additional variability is caused by the inherent statistical properties of the decay of radionuclides. This process has been shown [9] to follow the Poisson statistic, which is characterized by having the same mean and variance. The variability from the counting cannot be included in the *a priori* precision, because the number of recorded events is not known before the analysis is made, but unlike other *a posteriori* effects it can be calculated from a single measurement.

After taking into account the contribution from counting statistics it is possible to achieve agreement between the expected standard deviation and the observed variability of replicate results. Lack of agreement signals the presence of unknown sources of variability that usually give rise to systematic errors [11].

Bias

In accordance with the BIPM philosophy results must be corrected for all known systematic errors in order that they may be considered unbiased. However, the uncertainty of any correction must be included in the uncertainty budget. This means that systematic errors should have a mean value of zero, but a standard deviation different from zero, as is taken into account in Eq.(1).

Errors that contribute to the variability of results can be detected by their contribution to excess variance, but purely proportional or constant errors cannot be detected in this way. They are therefore particularly treacherous, and no effort should be spared to avoid them, most of all in certification analysis.

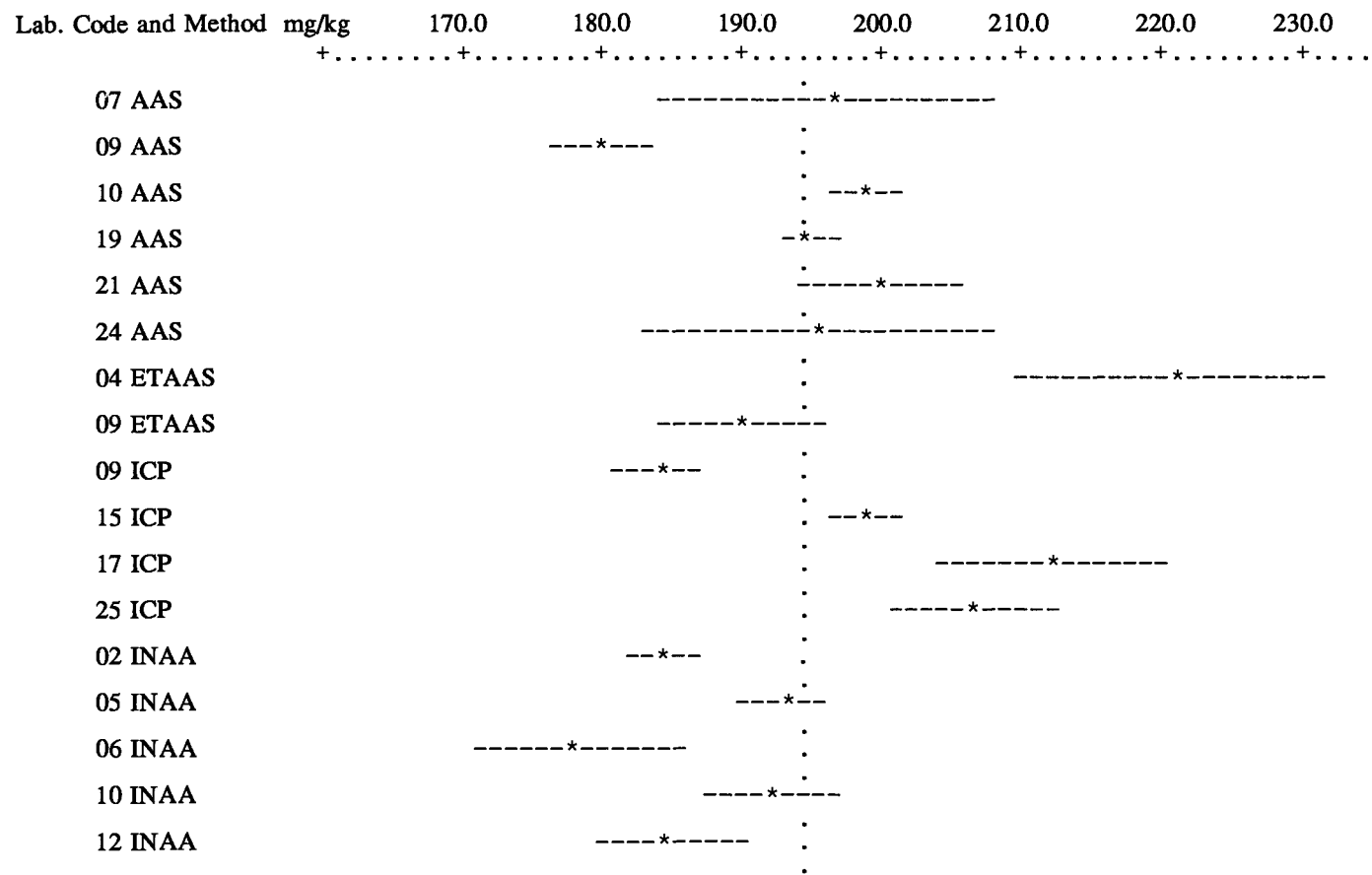


Figure 1: Certification analyses from 14 selected laboratories for manganese in CRM 182 steam coal by different methods

5.1.2 Advantages of NAA

In most analytical methods it is assumed that there is no loss of determinand during the treatment of the sample preceding the final measurement. Although such losses may be minimal, they are not zero, so that without correction all analytical results are biased!

In instrumental or radiochemical neutron activation analysis with an individual yield determination, the results are not biased, but the uncertainty of the yield determination must be reflected in the combined uncertainty of the results. In routine NAA it may be assumed that the chemical yield is constant, but in certification analyses it should be determined for each sample analysed, so that the variability of the yield is reflected in the variability of the results.

Another advantage of NAA is that the *a priori* precision is usually independent of the concentration. This is caused by the absence of a blank value and by the addition of an amount of carrier that is independent of the actual content of the determinand. All sources of variability are therefore unaffected by the concentrations apart from the actual counting process, which is determined for each individual sample.

The most important advantage is perhaps that it has been amply demonstrated that statistical control can be achieved in practical analytical work [12].

5.1.3 Pitfalls

In spite of the apparent simplicity of NAA it is often difficult to reconcile differences between results from different laboratories, such as those shown in Figure 1. Only by consistent, systematic study of each step of the analysis is it possible to pinpoint the origin of errors [13]. This can be done only by those involved in the actual analytical work.

The use of substances of ill-defined or unknown composition as comparator standards may lead to gross errors that are difficult to apprehend. Only pure elements or compounds with known stoichiometry [14] should be used. Reference materials, whether certified or not, should be used to verify the calibration, but not serve as calibrants. In certification analysis, in particular, traceability to another reference material is unacceptable.

Calibration based upon another element is possible in NAA, when the nuclear characteristics of the corresponding radionuclides are well known [15]; for certification analysis such indirect traceability is not generally accepted.

The use of comparators of the element to be determined compensates at the same time for many sources of systematic variation that would have to be calculated separately and whose uncertainty is not easy to judge. This includes coincidence corrections, as well as correction for counting geometry.

Identification errors

Failure to correctly identify all photopeaks in a spectrum may lead to serious systematic errors, because without such information it is impossible to ascertain the purity of a photopeak for the determination of a specific element.

An example of this is the unexpected presence of ^{147}Nd in a spectrum of a reference material to be certified for Cr [16], which might give rise to a systematic error of more than 10 %.

Interference from ^{75}Se on ^{203}Hg , ^{46}Sc on ^{65}Zn , is well-known and rarely overlooked, while interference from ^{152}Eu on ^{65}Zn is often disregarded.

Data processing errors

Photopeak area evaluation errors are closely related to the problem of correct identification of photopeaks, and most programs can determine the peak area of a reasonably large photopeak, if the photopeak is known to be pure. However, in INAA it is not easy to ascertain the purity of photopeaks, and computer programs for the resolution of doublets are of little value and may give highly misleading results [17].

Only if the channel numbers and half-widths of all energies contributing to a photopeak are accurately known, can a good program provide reliable results [18].

5.1.4 Sample homogeneity

The contribution from lack of homogeneity of the sample material to the combined uncertainty of the analytical result can easily be taken into account in Eq. (1), when the sampling constant is known [19]; in certification analysis it is usually specified to analyse a sample that is large enough to avoid any influence from such lack of homogeneity.

Actual samples may not be uniform, and in that case it is not possible to estimate their influence on the uncertainty of analytical results.

5.2 Methods of validation

Validation of an analytical method requires that

- a) all systematic errors are eliminated or corrected for
- b) all sources of variability are identified and their combination to the estimated analytical uncertainty accounted for
- c) analytical results are in statistical control

The key to validation is the last point, which expresses that the estimated and observed variabilities among n replicate analytical results be in complete agreement. This is tested by the statistic

$$T = \sum_1^n \frac{(y_1 - \hat{\mu})^2}{\sigma_1^2} \quad (3)$$

where $\hat{\mu}$ is the weighted mean of n individual results

$$\hat{\mu} = \frac{\sum_1^n \omega_1 y_1}{\sum \omega_1} \quad (3a)$$

and the weights

$$\omega_1 = \frac{1}{\sigma_1^2} \quad (3b)$$

When results are in statistical control the statistic T is closely approximated by a χ^2 -distribution with $n-1$ degrees of freedom.

For m duplicates ($n = 2$) these formulae are simplified

$$T = \sum_1^m \frac{(y_1 - y_2)^2}{\hat{\sigma}_1^2 + \hat{\sigma}_2^2} \quad (3c)$$

with m degrees of freedom.

In neutron activation analysis and other radioanalytical methods the variability of results cannot be explained from the *a priori* precision estimated from Eq. (1), because of the significant contribution from counting statistics.

It has been shown unequivocally [3] that, when the *a priori* variance estimate is compounded with the variance contribution from counting statistics, the resulting combined $\hat{\sigma}$ should be used in Eq. (3) without any significant change in the distribution of T .

Also for neutron activation analysis the statistic T is therefore a most suitable test for the presence or absence of significant unknown or unexpected sources of variability.

5.2.1 *Uncertainty budget*

In neutron activation analysis the uncertainty budget is an evaluation of the contribution from all possible sources of variability to the distribution of replicate analytical results. Sources of variability with no significant contribution need not be accurately accounted for, while the most significant sources of variability must be carefully evaluated, often by conducting a series of experiments to determine the standard deviation of the distribution.

Identification of all sources of variability in a particular analytical method is possible only for the professional practitioner of the art - the same kind of person that makes certification analysis.

Significant sources of variability may be associated with all stages of the analysis and may have to be studied separately for each of several types:

- Sample characteristics
- Irradiation conditions
- Radionuclide properties
- Radiochemical separation
- Counting conditions
- Data processing

When the uncertainty budget is complete, there are no unknown sources of variability, and statistical control is maintained under all conditions. In that case the distribution of the statistic T is indistinguishable from a χ^2 -distribution and well-suited for a continuous quality control. When this stage has been reached it is usually practical to pool all relative uncertainties $\hat{\sigma}_r^2$, all absolute uncertainties $\hat{\sigma}_a^2$, and obtain the following:

$$\hat{\sigma}^2 = \hat{\sigma}_a^2 + \bar{y}^2 \hat{\sigma}_r^2 + \sigma_c^2 \quad (4)$$

where σ_c^2 is the contribution from counting statistics.

However, Eq. (3) is applicable **only** if the individual results y_i and their corresponding standard deviations $\hat{\sigma}_i$ are correctly calculated! Validation of the computer programs used for the analysis therefore has to be ascertained **before** any serious attempts to validate the analytical method are begun.

5.2.2 Validation of computer programs

Ideally, the originator of a computer program should be responsible for the validation of his work, since he is usually the only person with access or competence to correct or modify the code.

In practice the user of a computer program is unable to obtain adequate information on the limitations of the code, and therefore he has to validate it for his own intended application.

In NAA the most important program is the photopeak area evaluation code, which calculates not only the peak area directly used for the analytical result y_i , but also the associated standard deviation $\hat{\sigma}_i$ from counting statistics that may often be the most important contribution in Eq. (4).

All peak-area evaluation programs should be tested by means of standard reference spectra, generated with known peak areas of Gaussian shape and with a variability that is governed by the Poisson statistic. Oddly enough, such spectra are available only from the International Atomic Energy Agency (IAEA) [20]. These spectra consist of 6 replicates with $m = 22$ photopeaks with peak-to-base ratios varying by almost two orders of magnitude and with widely different precision. True values for 20 photopeaks have been published by the originators of these spectra. Unfortunately the spectra contain only 2048 channels of data, and the full widths at half maximum of all photopeaks are invariably less than 5.

Fundamental traceability

Traceability of a peak area evaluation program is now ascertained by calculating for each peak the statistic T from the 6 replicate spectra in accordance with Eq. (3) with $n = 6$ from the peak areas y_i and their standard deviations $\hat{\sigma}_i$.

In this case the distribution of T is expected to closely follow a χ^2 -distribution with 5 degrees of freedom, and this can be tested by the Kolmogoroff-Smirnov test [21].

This procedure utilizes the deviations d between the cumulative observed frequencies of T and the cumulative chi-square distribution curve for 5 degrees of freedom. The ogives for three different programs are shown in Figure 2, and their maximum deviations from the theoretical frequency are indicated.

The outcome of the test is presented in Table 1 together with the probabilities of exceeding the observed deviation for the three programs. Clearly, results for program 3 are not random samples from the chi-square distribution; consequently the method is not in statistical control, and the program is not validated.

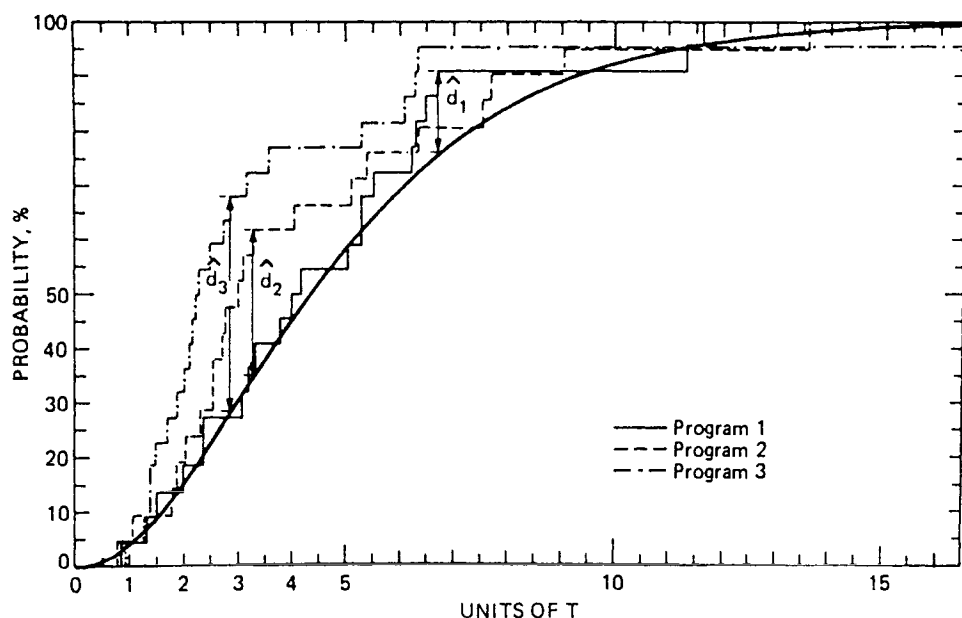


Figure 2: Cumulative distribution of values of T for three different programs compared with the chi-square distribution for 5 degrees of freedom.

Table 1: Kolmogoroff-Smirnov Test of cumulative distributions

Program	\hat{d}	m	p(d=0)
1	0.154	22	>0.10
2	0.276	21	>0.05
3	0.405	22	<0.01

Once a program gives results with reliable standard deviations, deviations from the true values can be tested for significance, and the absence of significant bias can be ascertained, using the statistic

$$X = \sum_1^m \frac{(\hat{\mu}_j - \mu_j)^2}{\sigma_j^2} \quad (5)$$

where $\hat{\mu}_j$ is the weighted mean of six replicates of each photopeak relative to the comparator, according to Eq. 3a; μ_j are the true ratios given by IAEA for each peak.

$$\frac{1}{\sigma_j^2} = \omega_j = \sum_1^6 \omega_{ij} \quad (5a)$$

where ω_{ij} is calculated according to Eq 3b.

This weighted sum of the squares of the deviations from the true values follows a chi-square distribution with m degrees of freedom, and for $m = 20$ the selected reference program no. 1 yields $X = 16.68$ with $p(\chi^2 \geq X) = 0.67$.

Practical traceability

Transfer of traceability from the fundamentally correct IAEA standard reference spectra to actual experimental spectral data is possible only after additional validation of the peak area evaluation programs.

The increase in the number of channels from 2000 to 8 K or even 16 K may be considered to be a trivial change, but the increase in the width of the photopeaks from a few channels in the standard reference spectra to 5 and upwards in practical, experimental spectra will alter the operation of most peak-area evaluation programs.

Practical reference spectra with a variety of photopeaks and a range of Full Widths at Half Maximum (FWHM) may be obtained by replicate countings of a fixed long-lived reference source at various gains. With an ^{152}Eu reference source 14 different photopeaks can be studied under replicate countings in 8192 channels at FWHM ranging from 2.5 to 15 channels, while the gain is being reduced from 0.1 to 0.4 keV/channel.

The influence of the half-width on the calculated photo-peak areas and their corresponding standard deviations is now determined by the T-statistic according to Eq. 3 for n replicates with different FWHM. As an example, results for our reference program are shown in Table 2, where all peaks are normalised to the largest and narrowest photopeak at 122 keV.

With the possible exception of the peak at 344 keV, which also has the lowest estimated standard deviation of 0.5 %, all other peaks are in good statistical control with $T = 268.2$ at 259 degrees of freedom, corresponding to $P(\chi^2 \geq T) = 0.34$. Results for the 344 keV peak were subjected to correlation analysis with respect to FWHM ranging from 3.0 to 13 channels, but with $r < 0.1$ no significant correlation was detected. Thus, for the Reference Program no systematic errors were found to be associated with changes in peak width, and statistical control was maintained down to less than 1% relative standard deviation.

Some programs have problems maintaining statistical control outside a certain range of FWHM, while others may produce photopeak areas that are correlated with the width of the peak [22].

With a reference program yielding results in statistical control within the entire range of FWHM encountered in actual practice, the validation of the program is transferred from the IAEA Standard Reference Spectra to actual spectra obtained in neutron activation analysis.

In fact, other peak-area evaluation programs may now be validated by comparison with the Reference Program [23]. Most commercial programs are capable of yielding unbiased peak areas, but tend to underestimate the corresponding standard deviation. This means that the statistic T may indicate the presence of unknown sources of excess variability, when in fact there are none.

Table 2: Verification of the precision of normalized results from a Reference Peak Evaluation Program for replicate countings at FWHM ranging from 2.8 to 15 channels

γ -energy keV	Total peak area counts	Number of replicates	Statistic T
245	43440 \pm 72	29	23.23
344	103578 \pm 95	29	45.95
368	3166 \pm 38	29	26.29
411	7281 \pm 34	29	22.02
444	9244 \pm 39	29	27.40
689	1673 \pm 30	29	23.07
779	21074 \pm 52	27	30.55
867	6150 \pm 44	23	25.25
964	19102 \pm 49	19	29.64
1112	15362 \pm 48	17	25.66
1213	1505 \pm 25	14	10.58
1299	1629 \pm 19	13	8.53
1408	19319 \pm 45	13	16.01
All energies		300	314.18

5.2.3 *Validation of the calibration function*

After all sources of variability have been brought in a state of statistical control only the calibration function need be validated. Neither a constant bias nor a biased slope of the calibration curve could be detected by generating any excess variability of the analytical results. Validation therefore has to be based on the determination of an accurately known quantity of a primary comparator material directly traceable to the pure element to be determined.

The inherently linear calibration function ascertains validation by using a single elemental comparator for each element for converting counts to quantity. Simultaneous determination of many elements therefore requires a multi-element comparator standard in order to maintain traceability. Such standards are notoriously difficult to prepare and maintain, and their validity must therefore be constantly ascertained.

Clearly the validation of an analytical method for certification analysis cannot be based on the analysis of other certified reference materials. Instead frequent preparations are required of fresh comparator standards from the pure element, a pure stoichiometric compound, or a certified elemental reference standard. Significant, unexpected changes in the calibration function are readily detected by comparing actual elemental ratios with ratios calculated from published k_0 -factors [24].

5.3 Practical validation

As a result of an uncertainty budget for the analytical method to be validated, we have identified the only significant contributions to the uncertainty of the final analytical result

irradiation exposure, and
counting statistics,

and we have calculated the standard deviation of the distribution of irradiation exposures around their mean from knowledge of the irradiation geometry [10,19].

In this case the combined uncertainty of a result $\hat{\sigma}$ is simply

$$\hat{\sigma}^2 = \sigma_{\text{irr}}^2 + \sigma_c^2 \quad (6)$$

where $\sigma_{\text{irr}}^2 = \bar{y}^2 \hat{\sigma}_r^2$ and $\hat{\sigma}_r \sim 2.5 \%$, while σ_c^2 is calculated by a validated computer program.

According to the budget - and reflected in Eq. (6) - the parameters and variables listed in Table 3 should have no direct influence on the uncertainty of the analytical result. This means that the data processing program should be capable of correcting accurately for all variables listed without introducing any variability that is not accounted for in Eq. 6; in other words the effect of all these variables is solely represented by the counting statistics.

Table 3: Factors and variables assumed to have no direct influence on the uncertainty of results by NAA

Method	Sample
Variables	
Irradiation time	Quantity
Neutron flux density	Activity
Decay time	Concentration
Counting time	γ -energy
Dead time	Half-life
Peak width	
Factors	
Detector	Determinand
Counting position	Matrix
Counting equipment	Indicator

The experimental validation of these assumptions and of Eq. (6) now has to be based on the verification of complete statistical control for real sample materials under all conceivable conditions of practical analysis, in order to show that variations in all these parameters are adequately accounted for. Since some of the corrections are not completely accountable, some of the variable parameters should not go beyond certain limits.

This applies particularly to corrections for losses during counting, which have to be corrected not only for deadtime, but also for the effects of pile-up at higher count rates. These corrections are verified by counting the same sample under such different conditions that both effects need different corrections. Most correction methods fail when there is a significant change of count-rate during the counting period, and therefore the relevant limitations of the method should be stated in the description of the method.

Highly standardized methods with fixed irradiation times, decay times, counting positions *etc.* result in almost constant corrections, which may very well be erroneous. Such methods therefore give reproducible, but biased results.

Reliable analytical methods are robust against changes in normal operating conditions and should not be rigidly standardized. In NAA it should be sufficient to specify the maximum permissible dead time during counting, and the irradiation and counting geometries that should be used.

5.3.1 *Experimental results used for validation*

The validation procedure proceeds stepwise through the following stages:

1. *Data processing*, checking the adequacy of corrections for decay, counting losses *etc.*
2. *Counting conditions*, checking the reproducibility of counting geometry for different materials at a full range of γ -energies
3. *Irradiation conditions*, checking the adequacy of the *a priori* estimate of variability among duplicates
4. *Radiochemical separation*, checking the adequacy of correction for losses during separation
5. *Sampling*, determining the variability among duplicate samples of relevant types of environmental materials

Validation refers only to the particular equipment and conditions specified in the analytical procedure and has to be repeated whenever the procedure is modified. The data reported in the following from the author's own laboratory serve merely to illustrate the method and to demonstrate that statistical control can be achieved in practice.

By allowing many factors and variables to change at the same time, but independently of each other, it is possible to validate Eq. (6) with a relatively small number of experiments.

① One sample of each of three different environmental materials should be irradiated and counted immediately after the end of irradiation for 2, 5 and 10 minutes in succession at a counting position giving a dead time close to, but not exceeding 25 %.

Using BCR certified reference materials for hay powder, fly ash and aquatic plant, irradiated for 10 s at a neutron flux density of 2.5×10^{13} n/cm²s, we were able to investigate the influence of:

decay	from 1 - 0.002
counting time	from 2 - 10 min
dead time	from 2 - 25 %
half life	from 2.2 - 900 min
γ -energy	from 0.62 - 2.75 MeV
determinands	Al, Br, Cl, K, Mn, Na
concentrations	70 $\mu\text{g.g}^{-1}$ - 35 mg.g^{-1}
matrices	3 different

Data from 3 successive countings were tested by the T-statistic in accordance with Eq. (3), and results are presented in Table 4 for each element and type of material.

With 2 degrees of freedom for each characteristic γ -line identified in all three countings, we find that for all three materials the variability of results for the elements determined could be entirely attributed to counting statistics. When all results are pooled, we obtain $T = 43.5$ for 40 degrees of freedom, which has a probability $P(\chi^2 \geq T) = 0.33$.

This means that corrections for decay, counting time and dead time, as well as pulse pile-up etc., do not give rise to additional variability of results within the range of conditions covered in the experiment. Longer half-lives give rise to smaller corrections, so that there is no need to make additional experiments with other determinands. Neither the elemental concentrations nor the matrix composition had any influence on the variability of results within the range of γ -energies covered by the experiment.

② Two samples of each of three different environmental materials should be irradiated together and counted alternately 1 hour after irradiation for 20 min and 1 h at the same counting position. Each pair was counted again in another position the following day.

Using BCR CRMs for cod muscle, aquatic plant, and beech leaves, irradiated between 10 s and 2 min, we were able to investigate the influence of:

irradiation geometry	$\hat{\sigma} = 2.5 \%$
decay	from 1 h - 24 h
counting time	from 20 min - 1 h
half-life	from 0.63 h - 35.3 h
counting position	76-257 mm from detector
precision	from 0.35-30 %
γ -energy	from 0.06 - 3.08 MeV
matrices	3 different

Data from duplicate countings in 4 different counting positions were tested by the T-statistic in accordance with Eq. (3c), and results are presented in Table 5 for each counting position, grouped according to the counting precision; results from ① are included for the corresponding counting positions.

Table 4:

Statistical control in triple countings* of three different environmental samples, corrected for dead-time losses and radioactive decay

Irradiated material and code		Fly ash BCR 38			Aquatic plant BCR 61			Hay powder BCR 129			
% dead time		start = 26% finish = 2%			start = 21% finish = 6%			start = 24% finish = 17%			
Radionuclide symbol	Half-life min	Number of γ -lines	Precision %	Statistic T	Number of γ -lines	Precision %	Statistic T	Number of γ -lines	Precision %	Statistic T	
^{28}Al	2.2	2	0.8	4.21	2	1.0	1.97	–			
^{56}Mn	155	1	2.5	1.84	3	0.5	8.65	2	0.7	10.89	
^{24}Na	900	–			1	8.5	5.06	2	1.0	3.34	
^{38}Cl	37	–			1	7.5	0.54	4	0.5	2.05	
^{42}K	722	–			–			1	3.2	1.77	
^{80}Br	18	–			–			1	2.1	3.14	
Degrees of freedom		$3 \times 2 = 6$ d.f.			6.05	$7 \times 2 = 14$ d.f.		16.22	$10 \times 2 = 20$ d.f.		21.19

* 2 min, 5 min, and 10 min

Table 5: Reproducibility of counting geometry in four different positions

Precision %	Position 257 mm		Position 175 mm	
	d.f.*	T	d.f.	T
< 2 %	12	13.15	14	18.25
2-8 %	15	17.46	7	8.56
> 8 %	17	11.72	7	5.46
Total $P(\chi^2 \geq T)$	44	42.33	28	32.27
	0.54		0.26	
Precision %	Position 117 mm		Position 76 mm	
	d.f.	T	d.f.	T
< 2 %	6	6.05	9	12.77
2-8 %	13	11.21	13	8.96
> 8 %	8	9.62	3	0.53
Total $P(\chi^2 \geq T)$	27	26.88	25	22.26
	0.47		0.62	

*Degrees of freedom = number of pairs of identified spectral peaks

In order to cover as thoroughly as possible the range of γ -energies, as well as different levels of precision, all unequivocally identified peaks present in duplicate spectra were used for the test, with the exception of

- single escape peaks, which - unlike double escape peaks - tend to be asymmetric [25], and therefore cannot be evaluated properly by the photopeak integration program.
- annihilation peaks, which also tend to be asymmetric, and whose intensity is determined by high-energy γ -lines with different half-lives, so that a correct decay correction is not possible.
- 1293 keV γ -ray from ^{41}Ar originating from the air in the irradiation container, and not from the sample.

Results in Table 5 demonstrate the absence of excess variability under all conditions of the experiment, even at the best precision. With a total $T = 123.74$ at 124 d.f. the reproducibility of counting geometry in all 4 positions investigated does not contribute to the uncertainty for any of the 5 types of environmental materials included in the investigation.

③ Two large samples of an environmental material with a very high degree of homogeneity should be irradiated together and counted in the same counting geometry after several days of decay. A sub-sample of approx. 10 % should be taken of each sample and counted in the same counting position for a correspondingly longer period.

Using a BCR certified reference material of river sediment [26] with a minimum recommended sample size of 100 mg, we irradiated one-gram samples for 3 hours in a thermal neutron flux density of 4×10^{13} n/cm²s. After a decay of 10 days each sample was divided into approximately 900 and 100 mg in separate counting vials, and counted for 30 min and 5 h respectively. This enabled us to check the influence of:

irradiation geometry	$\hat{\sigma} = 2.5 \%$
sample size	from 0.1-0.9 g
counting position	26-257 mm from detector
counting time	from 30 min - 5 h
half-life	900 min - 5.3 y

Results in Table 6 for 2 sets of samples at 2 different counting positions are shown to be independent of sample size at these two counting geometries, when compared by Eq. 3c at a counting precision of less than 1 %. For the element Sc the high value of T shows that results are not identical at a level of 0.3 %, indicating an uneven distribution among the samples in spite of its status as certified; the same lack of agreement could be observed for other trace elements for which no certification was attempted.

Table 6: Comparison of results obtained for 100 mg and 900 mg samples of river sediment CRM 320 after 1-2 weeks' decay

Element symbol	Concentration mg/kg	Relative precision	Number of comparisons	Statistic T
Fe	40 000	0.6 %	6	7.06
Ca	20 000	7.5 %	4	6.01
Na	17 000	1.1 %	6	4.75
Rb	150	5.5 %	4	1.35
Cr	138*	1.4 %	4	4.99
Co	17	1.7 %	6	6.07
Cs	5	6.5 %	8	7.48
$P(\chi^2 \geq T) = 0.48$ for degrees of freedom = 38				37.71
Sc	15*	0.3 %	8	28.68
$P(\chi^2 \geq T) < 0.03$ for degrees of freedom = 46				66.39

*Certified value

Verification of the first part of the variance contribution in Eq. (6) is done by comparing results from pairs of samples irradiated together. This must be done with the smallest possible uncertainty and is based on pooling the ratios for all photopeaks for elements assumed to be homogeneously distributed in the materials used, in particular those found in the upper part of Table 6.

Results for pairs of samples from 4 different reference materials counted in 6 different counting positions are shown in Table 7. For each pair a mean ratio and standard deviation is calculated using Eq. 3a and 3b, and the T-statistic is calculated in accordance with Eq. (3). With $T = 79.13$ at 74 degrees of freedom, a probability of $P(\chi^2 \geq T) = 0.32$ ascertains that all ratios were unaffected by possible sample heterogeneity, but only by differences in exposure to neutrons.

The distribution of ratios larger than one between samples from a normal distribution with a relative standard deviation σ_r has a mean of $1 + \frac{2\sigma_r}{\sqrt{\pi}}$ and a variance $\sigma_r^2 (2 - 4/\pi)$; for $\sigma_r = 2.5\%$ this gives a mean of 1.028 and a standard deviation of 0.021, in very good agreement with the mean and standard deviation calculated from the data in Table 7.

Table 7: Ratios of γ -intensity between pairs of samples of reference materials irradiated simultaneously

Counting position	BCR Code	d.f.*	T	Ratio \pm SD
257 mm	CRM 61	8	8.16	1.069 \pm 0.003
175 mm	CRM 422	8	7.90	1.045 \pm 0.003
117 mm	CRM 100	10	13.59	1.024 \pm 0.003
-	CRM 320	8	11.07	1.019 \pm 0.010
76 mm	CRM 61	11	9.39	1.050 \pm 0.007
-	CRM 320	9	11.20	1.007 \pm 0.004
47 mm	CRM 422	14	12.95	1.047 \pm 0.003
26 mm	CRM 100	6	4.87	1.028 \pm 0.011
All positions and materials		74	79.13	1.036 \pm 0.007

*Degrees of freedom = number of pairs of spectral peaks used
in the calculation of the ratio

A better test is obtained by applying the T-statistic to the ratios in accordance with Eq. (3). In this case we take $\hat{\sigma}_i^2 = 2 \sigma_r^2$ and $(y_i - \hat{\mu})^2 = (\text{Ratio} - 1)^2$ and propagate the standard deviations of the individual ratios to the sum of squares

$$\sum_1^8 (\text{Ratio}-1)^2 = 0.0132 \pm 0.0012$$

$$T = \frac{0.0132 \pm 0.0012}{2 \cdot (0.025)^2} = 10.56 \pm 0.92$$

which gives acceptance of Eq. (6) at

$$P(\chi^2 \geq T) = 0.23 \pm 0.06$$

④ There is no simple way of directly checking the complete equilibrium between the determinand in the sample and the added carrier used for the determination of chemical yield. The breaking of chemical bonds by neutron capture, usually referred to as the Szilard-Chalmer's reaction, eliminates the need for complete mineralization of the analytical sample to achieve carrier equilibration. Comprehensive neutron activation analysis is therefore unaffected by the chemical compound from which the determinand originates.

The only source of variation associated with the radiochemical separation is therefore the experimental determination of the chemical yield. This determination may be carried out in many different ways, including instrumental neutron activation analysis with an estimated standard deviation in accordance with Eq. 6. An actual example of those calculations for the determination of Cl by RNAA with re-irradiation yield determination is reported in the literature [27].

⑤ Simultaneous analysis of duplicate samples of an actual environmental material is a simple way of detecting excess variability. When all sources of analytical variation are in statistical control, all excess variability must be attributed to sampling problems [19]. Such problems are eliminated for reference materials by careful homogenization; how they are handled in real environmental studies is beyond the scope of this article.

5.3.2 *Verification and traceability*

When complete agreement between the experimentally observed variability of results and the standard deviation estimated from Eq. (6) has been ascertained under all practically occurring conditions, the analytical method is in a state of statistical control.

This means that each analytical result can be assigned a reliable standard uncertainty; this in turn makes it possible to compare results with known values in order to detect strictly proportional - calibration - errors or strictly additive - blank - errors. The aim is to ascertain that results are unbiased, which means that the mean value of a set of results $\bar{\mu}$ is not significantly different from the conventional true value $\hat{\mu}$ of a certified reference material or other well defined sample,

$$\bar{\mu} - \hat{\mu} \equiv 0 \quad (7)$$

with a high probability.

Such comparisons should cover the entire range of elements, concentrations and matrices encountered during an actual investigation; a number of certified reference materials is therefore needed to confirm the validity of Eq. (7) under all practical conditions.

An actual set of data for the determination of Se in a variety of biological matrices [11] is shown in Table 8.

Table 8: Results for Selenium in mg/kg by neutron activation analysis of a series of BCR biological reference materials

Certified Reference Material			Analytical result	Ratio
BCR Code	Type of material	Confidence interval	Mean \pm standard deviation	\pm standard deviation
CRM 184	Bovine muscle	183 ± 12	179 ± 3	0.98 ± 0.03
CRM 185	Bovine liver	446 ± 13	453 ± 27	1.02 ± 0.06
CRM 186	Pig kidney	10300 ± 500	10860 ± 170	1.05 ± 0.03
CRM 189	Wholemeal flour	132 ± 10	127 ± 3	0.96 ± 0.04
CRM 278	Mussel tissue	1660 ± 40	1647 ± 25	0.99 ± 0.02
CRM 279	Sea lettuce	593 ± 32	600 ± 22	1.01 ± 0.05
CRM 281	Rye grass	28 ± 4	22.2 ± 2.5	0.79 ± 0.11
			Weighted mean	1.00 ± 0.01
			T	8.17
			d.f.	6
			$P(\chi^2 \geq T)$	0.23

The 7 different certified reference materials cover a range of Se concentrations of more than two orders of magnitude, and the reported confidence interval is chosen at the 95 % level. Therefore, with a good approximation we obtain standard deviations by reducing with a factor of two.

The standard deviations of the analytical results are calculated from Eq. (6), which has to be applied to both the sample and comparator. The contribution from irradiation conditions is therefore multiplied by $\sqrt{2}$, whereas the counting statistics from the comparator is usually insignificant in comparison with the sample. In order to improve the precision all analyses were carried out in quintuplicate, so that the standard deviation of the mean was reduced by $\sqrt{5}$.

The ratio of the observed result to the certified concentration is shown in the last column together with the propagated standard deviations, and finally all these results were pooled in accordance with Eq. 3a and 3b. The weighted mean shows that the results are unbiased, and the T-test shows that this applies to the entire range of concentrations.

Determination of elements by NAA for which very few - or no - certified reference materials are available has to be validated indirectly by ascertaining the quality of the comparator standards. Simultaneous irradiation of several elemental comparator standards opens the possibility of checking their internal consistency by the calculation of k_c -ratios [24]. Replicate determinations of these ratios may serve to detect unexpected variability as well as significant bias by comparison with published k_c -factors [15].

Verification of the absence of bias and proportional errors by the use of CRM as well as by pure elemental standards completes the validation of the analytical method and provides the required traceability to internationally accepted standards.

5.4 Conclusion

Neutron activation analysis is in many respects a very special method, and its validation is also rather special. The principles used for validation are, however, of a general nature:

The identification and quantification of all significant contributions to the variability of analytical results is necessary in order to achieve a state of statistical control, and the major effort in the validation is to ascertain that contributions from all other factors can be neglected.

The final stage of validation that demonstrates the absence of significant bias by the analysis of certified reference materials or synthetic samples with known composition is no different from other methods of analysis.

NAA is unique because it is traceable to a weighed quantity of a pure element; other methods may therefore be validated by traceability to results by neutron activation analysis.

Acronyms

BIPM	Bureau Internationale de Poids et Mesures
ISO	International Organization for Standardization
NAA	Neutron Activation Analysis
INAA	Instrumental Neutron Activation Analysis
RNAA	Radiochemical Neutron Activation Analysis
K	2^{10}
FWHM	Full Width at Half Maximum
CRM	Certified Reference Material

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6.

Flow-through (bio)chemical sensors in environmental analysis

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Setting a definition for a "(bio)chemical sensor" is far from easy. Ideally, a (bio)chemical sensor is an analytical device that responds in a direct, reversible, continuous, rapid, and accurate (precise) manner to changes in the concentration of chemical or biochemical species in an untreated sample. In short, the primary use of an ideal sensor is for integrating two of the three general steps of the analytical process (preliminary operations, measurement and transduction of the analytical signal). Thus, sampling, addition of reagents, detection, separation, *etc.*, need not be included. However, many of the sensors developed in the last few years fail to meet one or more of the above criteria.

The large number and variety of sensors reported so far warrant classification according to criteria including the monitored parameter (chemical or biochemical), the nature of the sensor (reversible, irreversible, disposable or reusable), its external shape (planar, probe-type, flow-cell), the relationship between the sensitive microzone and transducer (connected or integrated), the operational mode used (batch or continuous), the presence or absence of a (bio)chemical process (whether active or passive), inclusion or not of an additional separation process, the type of transducer used (optical, electrical, thermal, mass or otherwise), and the number of monitored species (a single or several parameters, whether individually or integrated).

A flow-through sensor is an analytical device consisting of an active microzone where one or more chemical or biochemical reactions, in addition to a separation process, can take place. The microzone is connected to or incorporated into an optical, electric, thermal or mass transducer and must respond in a direct, reversible, continuous, expeditious and accurate manner to changes in the concentrations of chemical or biochemical species in the liquid or gaseous sample that is passed over it, whether forcefully (by aspiration or injection) or otherwise (gases).

It is worth setting a clear distinction between continuous-flow analytical systems (sometimes referred to as "sensor systems"), probe-type sensors and flow-through (bio)chemical sensors. As shown in Figure 1, in continuous-flow systems, separations, which involve mass transfer between two phases, chemical reactions and continuous detection take place separately, in a sequential manner. In probe-type (bio)chemical sensors, separation and/or (bio)chemical reaction takes place on the sensitive microzone, which is connected to the transducer. However, in flow-through (bio)chemical sensors, separation and/or a derivatizing reaction are integrated with detection since the active microzone is located in the flow-cell. The external appearance of the sensitive microzone can be as widely varied as the type of detector and process concerned.

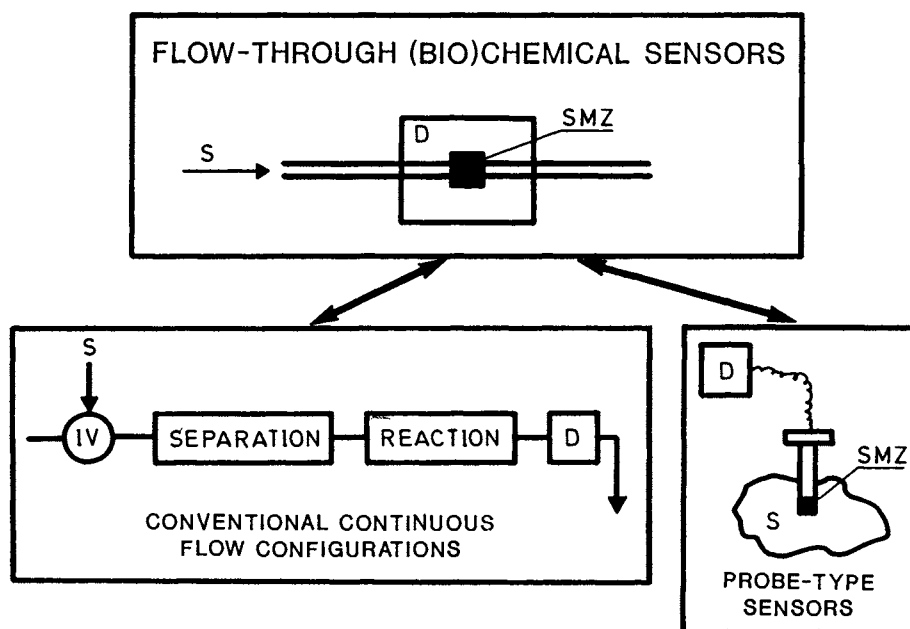


Figure 1: Flow-through (bio)chemical sensors compared with continuous-flow systems and probe-type sensors.

As shown in Figure 2, the flow-cell of a non-destructive optical detector can be used to immobilize, on a suitable support or even on the cell walls, any of the ingredients of a chemical or biochemical reaction, viz. the analyte, reagent, catalyst or some reaction product. The immobilization can be temporary (analyte or reaction product) or permanent (reagent or catalyst). These continuous-flow systems typically involve two major processes, namely,

retention/elution and reaction(s) (whether indicator, analytical or complementary), which are usually closely related to each other. The analytical reaction can take place at different positions along the manifold, *viz.* at the support located in the flow-cell when this retains the analyte or reagent; in the solution held in the flow-cell when it is the catalyst that is immobilized; and in the reaction coil when the reaction product is the immobilized species.

Depending on the type of detection involved and the process taking place at the active microzone (reaction and/or separation), the flow-cell that contains the microzone can exist in a variety of configurations [1], all of which are integrated with or connected to a measuring instrument in such a way that at least one of the above-mentioned processes takes place simultaneously with detection.

One of the most valuable assets of flow-through (bio)chemical sensors is their compatibility with unsegmented-flow configurations, which endows them with major advantages over probe-type sensors including higher flexibility and automatability in addition to a wider applicability to real rather than academic problems, the former being rarely addressed by using sensors.

A continuous configuration used in conjunction with a sensor can serve a variety of functions depending on the nature of the sample and analyte, the type of sensor used (*viz.* on the nature of the active microzone and immobilized species) and the type of integrated or connected detector: (a) transferring the injected or aspirated sample to the sensor; (b) conditioning the sample (pH adjustment, mixing with other reagents, masking) for optimal development of the process (reaction and/or detection) that is to take place at the active microzone; (c) maintaining or preparing the sensor for use; (d) regenerating the sensor between samples (or samples and standards) if it is of regenerable-reusable type; (e) facilitating straightforward, reliable calibration; (f) increasing the sensor selectivity and sensitivity via a continuous separation module; (g) boosting precision through reduced human participation in sensor-related operations; (h) extending applicability to other analytes giving rise to the same reaction products for which the sensor was originally designed; (i) allowing the development of kinetic methodologies based on differential rather than absolute measurements; and (j) filling the gap between sensor achievements and real analytical problems.

Many flow-through (bio)chemical sensors are irreversible-reusable and operate in two steps. In this context, regeneration can be defined as the operation following passage of the sample through the sensor by which the active microzone is made ready for the next sample by means of a (bio)chemical and/or physico-chemical process. As a result, the continuous signal provided by the sensor is returned to its baseline. Sensor regeneration may involve one or more of the following procedures: (a) flushing any residues of the previously analysed sample from tubes, connectors and the inside of the flow-through sensor itself; (b) removing any potential interferents that might have been retained at the active microzone; (c) eluting the temporarily immobilized species (analyte, reaction product); and (d) conditioning the active microzone, which may entail preparing the immobilized reagent or separation device (*e.g.* a membrane).

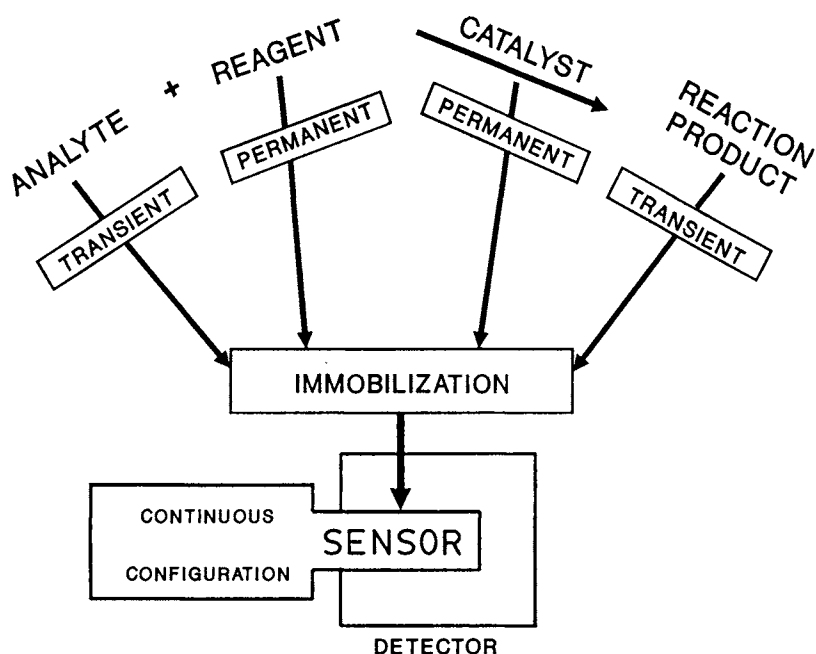


Figure 2: Types of immobilization in flow-through (bio)chemical sensors used in continuous systems and location where the main derivatizing reaction takes place.

The simplest procedure for regenerating flow-through sensors involves injecting an unusually high volume of sample (between 0.3 and a few ml) directly into the regenerating carrier (*e.g.* see [2]). The sensor is placed near the injection valve in order to avoid dispersion (mixing) of the injected macro plug, which reaches the sensor as such or after mixing with a reagent stream. The procedure is most convenient as it is performed immediately after the injected macro plug passes through the sensor and no additional operation is required since the carrier acts both as such and as regenerator.

One alternative regenerating procedure involves the sequential aspiration of the sample and regenerating carrier, which requires actuating the switching valve in order to restore the sensor. It allows larger sample volumes to be used in order to raise the analyte concentration at the active microzone when highly dilute samples are to be processed (*e.g.* see [3]).

If the sample has to be conditioned, it is mixed with the carrier in a coil before they are transferred to the sensor, which is regenerated at a later stage by introducing the regenerating carrier by injection or aspiration *via* a switching valve. The carrier is passed through the sensor over a given interval during which the maximum signal provided by the injected sample is recorded.

One essential feature of flow-through sensors is the response (transient signal) they provide *via* the instrument (detector) to which they are connected or in which they are accommodated. Such a response is typically a transient signal similar to those provided by other systems including Flow Injection (FI) configurations, (bio)chemiluminescence detectors and electrothermal vaporization atomic absorption spectrometers, but different from the steady-state signals afforded by probe sensors. The shape of such transient signals depends on (a) whether the sensor is reversible or irreversible (reusable), (b) the type of process taking place at the active microzone (separation, reaction or both), (c) which species is immobilized, (d) the type of detector used, and (e) the operational mode in which the continuous configuration coupled on-line to the sensor is employed.

The transient signals provided by flow-through (bio)chemical sensors can be measured in various ways in order to draw information that can be directly related to the analyte concentration in the sample. Figure 3 shows the more frequently used approaches in this respect, classified according to whether they rely on direct (a) or kinetic measurements (b).

The most common procedure (Figure 3.A) involves measuring the transient signal provided by a reversible or irreversible-reusable sensor at the maximum and plateau obtained by injecting the sample into a non-regenerating carrier.

Kinetic measurements are based on signal increments over preset intervals and have the advantage of their relative rather than absolute nature, which avoids interferences from the sample matrix. Figure 3.B shows the different variants of kinetic measurements in this context, which depend on the type of sensor and coupled continuous configuration used. The most immediate variant involves halting the flow over an interval Δt when the sample plug reaches the detector (Figure 3.B.2), where the (bio)chemical reaction is allowed to develop while the product of interest is monitored simultaneously. The other two variants warrant more detailed discussion on account of their innovativeness and great interest and potential.

Kinetic *in situ* concentration methods (Figure 3.B.1) rely on measurements of the slope of the initial portion of the transient signal, which arises from passage of the sample plug through the detector (e.g. see [3]). The signal rises as the analyte accumulates on the active microzone. The signal increment over a preset interval is proportional to the analyte concentration in the sample. In common with every kinetic method, *in situ* concentration methods offer substantially increased selectivity. In addition, they avoid the need to wait for the signal maximum to be reached (*i.e.* for the whole injected or aspirated sample macro plug to pass through the reactor) since the signal increment over a preset interval soon after injection is more than adequate for measurement purposes; in this way, the sample throughput can be doubled or trebled as a result. Microprocessors for signal acquisition and processing make this an affordable choice for routine analyses of vast numbers of samples. In addition, kinetic *in situ* concentration methods avoid the two most severe shortcomings of conventional preconcentration methods, *viz.* slowness and the need to use large sample volumes.

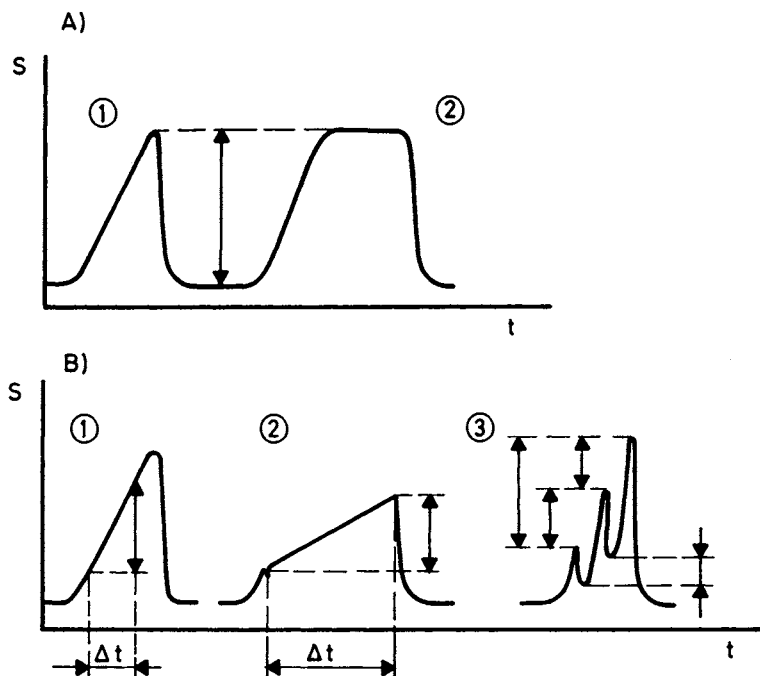


Figure 3: Types of measurements available on the transient signals provided by flow-through (bio)chemical sensors. (A) Ordinary measurements. (B) Kinetic measurements. For details, see text.

The multippeak recordings obtained by passing the sample plug many times through the detector or using an open-closed circuit can be used to draw valuable information in the form of various types of measurement (*e.g.* see [4,5]). Thus, signal increments can be measured between peak maxima or minima at fixed intervals, so they can also be considered kinetic measurements. Alternatively, individual kinetic measurements can be added up in order to boost the sensitivity of the flow-through sensor concerned, which usually arises from inclusion of a permanently immobilized reagent in the active microzone.

In dealing with the mechanism of action (and response) of a flow-through (bio)chemical sensor, one should consider three different types of kinetics, namely: (a) physical kinetics, which arises from the intrinsic dynamic character of flow-through sensors and is essentially related to the transfer of sample, reagent and regenerating carrier to the sensor microzone; (b) physico-chemical kinetics, which is basically related to separation processes that take place at the sensor microzone (transfers of analytes or reaction products across separation membranes to the surface or inside of a sorbent material until a partitioning equilibrium is reached); (c) chemical kinetics, which is associated with the chemical reaction that takes

place simultaneously with detection and sequentially or simultaneously with a separation process at the active microzone. With the exception of flow-through sensors used for reaction-rate measurements, the processes taking place at the active microzone of a flow-through sensor (transport, separation and reaction) must be rapid enough for the transient signal to be as short-lived as possible.

Flow-through sensors should therefore meet four essential requirements for proper performance, namely:

- (a) They should be fully reversible (or readily and expeditiously regenerable otherwise). Hence single-use (*e.g.* probe and drop planar) sensors are of very little service here.
- (b) The type of immobilization used should be suited to each particular sensor. With permanent immobilization (reagent or catalyst), the physico-chemical linkage or chemical bond of the immobilized species to the support must be highly stable under the sensor operational conditions. With sensors based on a transiently immobilized analyte or reaction product, chemical or physico-chemical retention must be very strong in order to avoid sweeping by the sample or conditioning carrier, but also readily and rapidly overcome on passage of the regenerating carrier through irreversible-reusable sensors.
- (c) As noted earlier, the physical, physico-chemical and (bio)chemical kinetics inherent in on-line coupled biosensors and continuous configurations must be fast enough for the sensor to perform properly;
- (d) The active microzone and sensing system used must be mutually compatible, *i.e.* the detector must be responsive to changes in the microzone, which in turn must not alter the detector functioning. Such compatibility is reflected in the obtainment of a stable baseline (close to the zero signal level) in the absence of sample and a perceptible rise on its passage through the detector. The above four requirements vary with the type of flow-through sensor and detection system used.

Despite their high potential for solving real problems, flow-through (bio)chemical sensors have scarcely been used in the environmental field. Accordingly, this chapter discusses applications developed by using not only flow-through (bio)chemical sensors specially designed for each purpose, but others used in clinical analysis that can be employed as such or slightly altered for environmental applications. For clarity, the sensors described are classified in terms of the step(s) integrated with detection; thus, sensors in which the chemical reaction takes place in the flow-cell are discussed first (section 1), followed by those in which separation and detection are simultaneous (Section 2) and, finally, those that integrate reaction, separation and detection (Section 3).

6.1 Flow-through (bio)chemical sensors based on integrated reaction and detection

Flow-through sensors integrating detection and a chemical or biochemical reaction use a chemical species that takes part in (reactant) or catalyses (catalyst) the reaction by which the analyte is measured; such a species is immobilized in the probe, the proper flow-cell or a special cell housing. Some sensors containing an immobilized reactant are of great interest and wide usage that is bound to increase even further in the near future. The presence of a biological material (usually an enzyme, but also some biomolecule or tissue) allows the analytical reaction to be completed in a fairly short time. These are the so-called "biosensors", the current significance of which reflects in the vast amount of published material on the topic [6–14]. While less common, sensors using a non-biological catalyst are also of some interest. Sensors incorporating an immobilized reactant can obviously be divided into those where the reactant is consumed and those where it is recycled; this classification, logically, does not apply to sensors using an immobilized catalyst.

Sensors including a catalyst immobilized on the sensing surface, the flow-cell or specially designed cell housing are particularly effective in those cases where the analytical reaction is integrated with measurements on account of their reversible character. Catalysts taking part in (bio)chemical reactions allow the development of ideal reversible sensors of potentially unlimited use. In practice, the nature of the sample and/or the medium where the derivatizing reaction takes place, in addition to the hydrodynamic features of the flow system and the nature of the catalyst itself, limit the use of reusable sensors to a greater or lesser number of samples or even a single one (disposable sensors).

One essential difference between flow-through sensors including an immobilized catalyst arises from the nature of the catalyst: biochemical (an enzyme, usually) or chemical (generally inorganic). The former are much less common and widely used than the latter.

6.1.1 *Sensors based on an immobilized biocatalyst: biosensors*

The term "biosensor", in common with those used to denote devices of scientific and non-scientific interest, has been the subject of a number of definitions of both a generic and specific nature. In broad terms, a biosensor is any instrument for measuring biomolecules. On the other hand, Rechnitz defines sensors in a stricter sense as "devices that incorporate a biochemical or biological component as a molecular recognition element and yield an analytical signal in response to biomolecules" [15]. In between these two extreme definitions, a biosensor can be considered as "a system of two transducers, biochemical and physical, in intimate contact with each other, which relate the concentration of an analyte to a measurable electric signal". As a rule, a biosensor is a sensor used for measuring biological species in which both the species that facilitates measurement and the analyte or only the former are of a biological nature, depending on which definition is adopted.

The current status of flow-through sensors based on immobilized enzymes is the result of intensive research in the 1980s and early 1990s. There is an ongoing search for new types of supports [16] and immobilization modes [16–18]. The wide use of immobilized enzymes in continuous-flow configurations [particularly flow injection analysis (FIA) manifolds] [19,20]

is a natural consequence of the comprehensive knowledge available on the behaviour of immobilized enzymes in dynamic systems. The ease with which samples can be treated prior to detection in such systems allows processing of complex samples requiring multi-step conditioning treatments (e.g. dialysis, pre-electrolysis, an enzymatic reaction [21]) for reliable measurement, as well as multideterminations [22].

Preliminary work in this area included both metabolic enzyme reactions (*i.e.* those where the substrate is consumed to form a product) and bioaffinity reactions (*i.e.* those where measurements are based on electron density changes). The latter are especially commonplace in clinical chemistry, where most substrates are of a biological nature.

Groom and Luong developed a flow-through biosensor for cyanide consisting of a column of rhodanase immobilized on a pre-activated nylon membrane and attached to the surface of an hydrogen peroxide electrode [23]. Immobilized rhodanase converted CN^- to SCN^- in the presence of $\text{S}_2\text{O}_3^{2-}$ and the SO_3^{2-} formed was converted to SO_4^{2-} in the presence of sulfite oxidase. The H_2O_2 produced was determined electrochemically at a Pt electrode vs Ag/AgCl. The calibration graph was linear from 5 to 1000 $\mu\text{mol.l}^{-1}$ cyanide. The results obtained compared well with those of soluble enzymic and colorimetric assays, but not with those of the commercially available Orion cyanide electrode.

Karube *et al.* constructed a special microbiosensor for the determination of CO_2 using micromachining techniques. One such sensor is based on a micro Clark-type oxygen electrode [24] made by photolithography and anisotropic etching for disposable use. Prior to this, they built a micro-oxygen electrode formed on a 2×15 mm silicon chip with a V-shaped groove (formed by anisotropically etching the silicon), a gold cathode and a gold anode. The electrodes were formed over an SiO_2 layer that provided electric insulation between them. The groove was filled with calcium alginate gel containing a 0.1 mol.l⁻¹ potassium chloride aqueous solution as the electrolyte and covered by the gas-permeable membrane. The oxygen concentration was measured as the reduction current on application of a constant voltage between the two electrodes. This electrode was used as the basis for the CO_2 , which was constructed by immobilizing autotrophic bacteria and used the same principle as the microbial sensor including a conventional galvanic oxygen electrode [25]. If a sample solution containing the CO_2 was added to the buffer solution into which the sensitive area of the sensor was immersed, the CO_2 permeated the membrane, reached the bacteria, and was assimilated. Bacterial respiration increased and more oxygen was consumed as a result. The change in the oxygen concentration inside the gas-permeable membrane was detected as an oxygen reduction current.

The biochemical oxygen demand (BOD) is one of the most widely used tests in the management of organic pollution. The conventional BOD test, however, involves a 5 day incubation period. Therefore, a more rapid and reproducible method is required for assessing BOD. *Trichosporon cutaneum*, which is used for waste water treatment, was employed to develop a BOD sensor. The dynamic system where the sensor was used consisted of a 0.01 mol.l⁻¹ phosphate buffer of pH 7 saturated with dissolved oxygen which was transferred to the flow-cell at a rate of 1 ml.min⁻¹. When the current reached a steady-state, a sample was injected into the flow-cell at a rate of 0.2 ml.min⁻¹. The steady-state

current was found to be dependent on the BOD of the sample solution. The current of the microbial sensor gradually returned to the initial level after the sample was washed out. The response time of the microbial biosensors depends on the nature of the sample solution. The difference between the initial and final (steady-state) current was found to be linearly related to BOD up to a standard concentration of 60 mg.l^{-1} in 5-day assays. The minimum measurable BOD was 3 mg.l^{-1} . The current is reproducible within 6% of the relative error when a BOD of 40 mg.l^{-1} is employed over 10 experiments [26].

Flow-through sensors for measuring oxygen and hydrogen peroxide can be constructed on the basis of many of the underlying principles of glucose sensors relying on the substrate oxidation, catalysed by the enzyme glucose oxidase. In this sense, luminescence techniques (e.g. fluorescence quenching by oxygen [27] and the formation of chemiluminescent compounds by using the H_2O_2 /peroxidase system [28]), in addition to colorimetric and electrochemical methods, lend themselves readily for application by use of this type of sensor.

Several methods exploiting the inhibitory effect of some analytes have been developed by using an enzyme thermistor for measuring the effect of a pollutant or enzyme on cell metabolism [29]. Urease inhibition has also been used for the determination of heavy metals including Hg(II) , Cu(II) and Ag(I) , with the enzyme immobilized on CPG. The response obtained for a 0.5-ml standard pulse of urea in phosphate buffer at a flow-rate of 1 ml.min^{-1} is registered, after which 0.5-ml of sample is injected. A fresh 0.5-ml standard pulse of urea is injected 30 s after the sample pulse (accurate timing is mandatory here) and the response compared with that of the non-inhibited peak. After a sample is run, the initial response can be restored by washing the column with $0.1\text{--}0.3 \text{ mol.l}^{-1}$ NaI plus 50 mmol.l^{-1} EDTA for 3 min. Under the reported conditions, 50 % inhibition (50 % of the initial response) was obtained for a 0.5-ml pulse of $0.04\text{--}0.05 \text{ mmol.l}^{-1}$ Hg(II) or Ag(I) , or 0.3 mmol.l^{-1} Cu(II) . The enzyme inhibition was irreversible in some cases. In this situation, a reversible enzyme immobilization technique that allowing easy replenishment of enzyme is required. This was accomplished by reversibly binding cholinesterase to a concanavalin A-Sepharose column [29]. The old enzyme was removed by injecting a pulse of 0.2 mol.l^{-1} glycine/HCl at pH 2.2. Fresh enzyme was then immobilized on the column simply by injecting the enzyme preparation while the column was in the enzyme thermistor apparatus.

6.1.2 *Sensors based on an immobilized non-biological catalyst*

The use of non-enzymatic catalysts for analytical purposes is limited by (a) the low selectivity of such catalysts relative to biological catalysts as a result of their lack of protein-selective or specific sites—they occasionally act on many substances of more or less similar nature—; and (b) the fact that substances of similar nature have similar effects to those of the catalyst. Overcoming both drawbacks in order to ensure a selective catalytic reaction entails isolating the substrate from the catalyst, which severely limits its utility. However, because this type of catalyst is usually less expensive than are biological catalysts, reusability is not a major concern here.

The effect of fluoride ion on the electrochemical behaviour of a zirconium metal electrode was used by Pihlar and Cencic [32] to develop a sensor for this halide. Because metal zirconium is always covered by a passivating oxide layer, the anodic features of a Zr/ZrO_2 electrode depend markedly on the electrolyte with which it is brought into contact. Only HCl and HClO_4 were found to ensure a proportional relationship between the fluoride concentration and the anodic current density; other electrolytes resulted in the fluoride-induced dissolution of metal zirconium and hence increased the thickness of the ZrO_2 film, thereby further hindering mass transfer of the halide through the oxide layer. The mechanism for the electrode dissolution of zirconium at low fluoride concentrations (below $1 \times 10^{-3} \text{ M}$) seemingly differed from that acting at low concentrations; in any case, the relationship between the equilibrium potential and the fluoride concentration deviates markedly from Nernstian behaviour. A thin layer cell including this type of electrode was used for the determination of nanogram amounts of fluoride in a flow system [32].

The electrooxidation of hydrazine is also catalysed by metal compounds other than oxides, as shown by Hou *et al.* [33], who used an FI manifold for determining the analyte. By using a cobalt/tetraphenylporphyrin-modified glassy carbon electrode, they accomplished the oxidation of hydrazine at pH 2.5 and +0.5 V vs Ag/AgCl , with a detection limit of 0.1 ng.

The electroreduction of typically inorganic compounds such as nitrogen oxides is catalysed by osmium polymer complexes including $[\text{Os}(\text{bipy})_2(\text{PV})_{20}\text{Cl}]\text{Cl}$, where bipy denotes 2,2'-bipyridyl and PVP poly(4-vinylpyridine). This polymer alters the reduction kinetics of nitrite relative to the reaction at a bare carbon electrode, thereby providing calibration curves of slope $0.197 \text{ nA cm}^3/\mu\text{g}$, a detection limit of $0.1 \mu\text{g.ml}^{-1}$ and excellent short-term reproducibility ($\text{rsd} = 2.15 \%$ for $n = 20$). No deterioration of the sensor performance was observed after three weeks of use in a flow system, during which 240 standards and 30 complex samples were injected [34].

Mottola *et al.* developed two types of electrodes modified with reversible $\text{Fe(II)}/\text{Fe(III)}$ sites for determining nitrogen oxides. One is a slightly altered version of a previously developed sensor made by mixing carbon paste with tris-4,7-diphenyl-1,10-phenanthroline/iron(II) perchlorate; the other is a glassy carbon surface electrode modified by oxidative electropolymerization of tris[5-amino-1,10-phenanthroline]iron(II) perchlorate. Both were used in flow systems. Continuous "bathing" of the sensor surface with supporting electrolyte ensures the presence of an unbroken film of ions to support the electrical migration and satisfy the electroneutrality requirement. The polymer-coated sensor compares favourably with the chemically modified carbon paste; in addition, it is highly resistant to poisoning, has a competitive detection limit (*ca.* 2 ng.ml^{-1} at +1.0 V vs Ag/AgCl) more than adequate selectivity towards NO_2 when used in a thin-layer cell, which allows up to 120 samples per h to be injected into a flow manifold. The typical concentration range amenable to determination is $2\text{--}25 \text{ ng.ml}^{-1}$, but depends on the thickness of the polymer film [35].

Wang and Liu used a thin film of polyaniline (PAn) deposited over an ordinary glassy carbon electrode by slow potential cycling ($20 \mu\text{V.s}^{-1}$) in $1.0 \text{ mol.l}^{-1} \text{ H}_2\text{SO}_4$ to develop a high stable sensor which they included in two continuous systems (flow-injection and ion chromatography) in order to determine iodide, bromide, thiocyanate and thiosulphate, the detection

limits thus achieved being 1, 5, 10 and 10 ng.ml⁻¹, respectively. The same CME surface was usable for cyclic voltammetry (CV) and FI experiments for 2 weeks or longer, with no evidence of chemical or mechanical deterioration. The CV response of the CME to various anions was also maintained at the same level no matter how often the electrode was exposed to other electrolytes during intervening CV scans. However, achieving a constant analytical response for several anions in FI systems entailed making some provisions for discharge of the anions accumulated in the course of repeated exposures. For a series of 20- μ l injections of 1.0×10^{-2} mol.l⁻¹ SO₄²⁻ at +0.6 V vs SCE, the oxidation peak currents measured in boric acid solution declined to only 50 % of the initial response after ten such exposures. However, the response decrease was much less severe at lower anion concentrations; also, for a series of 20- μ l injections of 1.0×10^{-4} mol.l⁻¹ SO₄²⁻, the response was 90 % of the initial level after ten exposures. High anion concentrations can thus be managed by using a discharge procedure previously developed for polypyrrole electrodes. The CME could be briefly exposed to a reducing potential of ca. -0.2 V between successive anion exposures. In this way, the polyaniline film was cycled through its fully reduced state with the accompanying discharge of any anions that had been incorporated as a result of polymer oxidation. One of the most salient and lesser studied features of this electrode is possibly the selectivity achieved by altering the composition of the electrolyte solution where the sample is injected [36].

6.1.3 Immunosensors

Immunosensors take advantage of the high selectivity provided by the molecular recognition of antibodies. Because of significant differences in affinity constants, antibodies may endow immunosensors with an extremely high sensitivity relative to enzyme sensors. In addition, antibodies against an unlimited number of determinants can in principle be obtained. Immunosensors are thus characterized by high selectivity, sensitivity, and versatility. These desirable features have fostered research and development of immunosensors in the last two decades, particularly in recent years, as the result of both the strong demand for immunoassays and remarkable advances in immunochemical technology, which has also benefitted from such ancillary technologies as optoelectronics.

Fiber optic immunosensors (FOIS) are the most desirable optical sensors inasmuch as they can in principle be used for both batch and continuous-flow measurements. However, the need for pre-incubation in practice, which can be avoided by using a suitable flow manifold—and the fairly sluggish response of many of these sensors preclude their use in continuous configurations. Little information has been published on homogeneous immunosensors despite the great endeavours involved in their development. The earliest homogeneous immunosensor was used for the determination of polynuclear aromatic hydrocarbons (PNAs) by their native fluorescence [37]; the poor sensitivity obtained—possibly arising from the small amount of immobilized antibody and denaturation on bonding to fibers—in addition to the long incubation (60 min) needed, made it unsuitable for use in flow-through configurations. However, later research into this sensor has provided some promising results [38].

Several amperometric immunosensors for the pesticide 2,4-dichlorophenoxyacetic acid (2,4-D) were developed by using a flow-through cell with the catching antibody covalently bound to a membrane of cellulose acetate or activated nylon (Figure 4). The hapten-molecule was conjugated with enzymes and the labelled and free antigens competed for the catching antibodies. Glucose oxidase (GOD) and alkaline phosphatase (AP) as marker enzymes yield signals at 0.6 and 0.1 V with glucose and 4-aminophenylphosphate, respectively, which are measures of the bound enzyme activity; this sensitivity is inadequate for pesticides, so it must be increased dramatically for application to environmental samples [39].

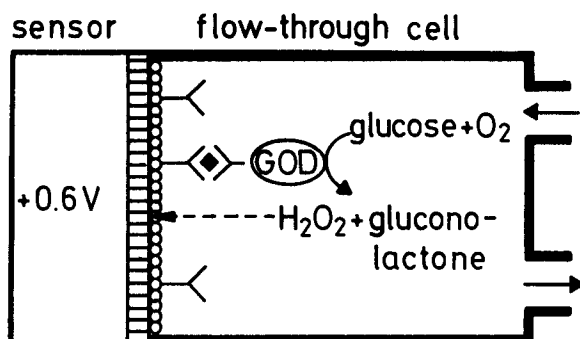


Figure 4: Principle of the membrane immunosensor. (Reproduced from [7] with permission of VHC Publishers).

Three different types of potentiometric immunosensor have been proposed. One is based on measurements of the transmembrane potential across an antibody (or antigen) membrane that specifically binds the corresponding antigen (or antibody) in solution. Concentrations of either the target antigen or antibody can be determined by measuring a change in the membrane surface. The second type relies on electrode potential measurements. The surface of an electrode is modified by an antibody or antigen that is capable of specifically binding the corresponding antigen or antibody. Immunocomplex formation causes the electrode potential to vary, primarily as a result of a change in surface charge related to the concentration of the analyte in solution. The third type of immunosensor is based on measurements of the surface potential at the gate of a field-effect transistor covered with a thin antibody binding membrane. Such a potential may vary with the concentration of the corresponding antigen in solution. Notwithstanding their high potential, these sensors have not yet been applied to environmental samples.

6.1.4 *Flow-through sensors based on consumption of the immobilized reagent*

Flow-through sensors using a non-regenerable immobilized reagent are largely unsuitable for continuous operation. The gradual loss of reagent from the sensitive microzone most often causes the signal to decrease as well, so adequate reproducibility is difficult to obtain in many cases. These sensors are usually based on luminescence techniques and are of integrated type since use of an optical fibre to hold the microzone on one end is impractical; the amount of reagent than can be immobilized in this way is so very small that the reagent uptake in each determination can give rise to sharp drops between successive signals.

Most sensors using a consumable reagent rely on chemiluminescence processes usually involving trichlorophenyl oxalate (TCPO) and a fluorophore (usually fluoranthene), or luminol.

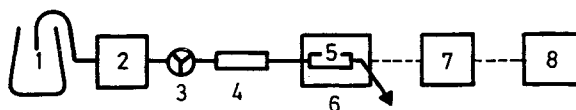
Frei and coworkers pioneered the development of flow-through sensors based on immobilized chemiluminescent reagents. Such sensors, based on the peroxyoxalate chemiluminescent system, were used for the determination of hydrogen peroxide [40]. The sensor was essentially non-regenerable since the reagent consumed (TCPO) was not immobilized in the flow cell, but in solid form in a previous reactor; the actually regenerated species was the fluorophore 3-aminofluoranthene, which was immobilized in the flow-cell as shown in Figure 5.A. Several procedures and supports for immobilizing the fluorophore were developed and the sensor was applied to the determination of H_2O_2 in rainwater (detection limit, $1 \times 10^{-8} \text{ mol.l}^{-1} \text{ H}_2\text{O}_2$). At a later stage, the sensor was altered in various ways to construct different variants lying between the sensors discussed in this and the next section inasmuch as they included an immobilized and a consumable reagent in the flow-cell. The dual-packed flow-cell schematically depicted in Figure 5.B allowed Frei *et al.* to place solid TCPO in the sensing zone and immobilize the luminophore on CPG, thereby forming two consecutive layers. The reproducibility thus achieved was excellent ($\text{rsd} = 3 \%$) and the log-log calibration plot obtained was linear over a range encompassing six orders of magnitude. While the sensor duration was not stated, the packing frequency must obviously have been a function of the analyte concentration in the samples assayed; in any case, each packing was followed by a new calibration owing to the irreproducibility of the packing procedure [41]. The reported sampling frequency (100 h^{-1}) is of little interest in relation to routine analyses taking into account the non-regenerable nature of the sensor.

Figure 5.C shows a modified version of the above-described sensors for biochemical measurements based on an enzyme catalyst (an oxidase) immobilized on CPG at a point prior to the detector [42].

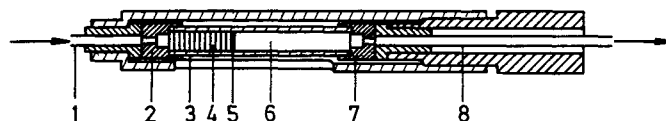
Hool and Nieman actually immobilized a reagent (luminol) in the flow-cell of a chemiluminescence detector by using silica and CPG as supports. For solubility reasons, the immobilization of luminol was carried out by using glutaraldehyde in an ethanol/dimethyl sulphoxide mixture. The loadings thus obtained were $29 \mu\text{mol luminol/g CPG}$ and $86 \mu\text{mol/g silica}$. No evidence was found of degradation of luminol to 3-aminophthalate. The immobilized material was packed into a flow-cell such as that depicted in Figure 5 and was inserted in a flow injection configuration. The slopes of the calibration curves for hydrogen peroxide obtained with both supports were close to unity. The log-log working curves for

peroxide were linear over the concentration range 20–600 $\mu\text{mol.l}^{-1}$, with a correlation coefficient $r = 0.998$ and a standard error of estimate of 0.047. Measurements of replicate injections (3–5 injections per data point) over the entire concentration range gave an rsd of 3 % for silica and 4 % for CPG. Inclusion of the bed of particles containing immobilized CL reagent resulted in somewhat degraded precision. Replacing the particles with dissolved luminol diminished the rsd to 1 %. Also, the peaks were 80 % broader in the presence of the luminol-loaded particles. HPLC analysis of the flow-cell effluent plus a comparison of chemiluminescence intensities of immobilized luminol and isoluminol showed the former to be hydrolysed from the support prior to or during the reaction, and emission to occur in solution. One other problem encountered arose from the use of glutaraldehyde as the linkage for luminol in the form of potential absorption by the particles of a significant portion of the chemiluminescence produced. An amount of 1 g of silica containing immobilized luminol was estimated to provide for over 500 peroxide assays [44].

A)



B)



C)

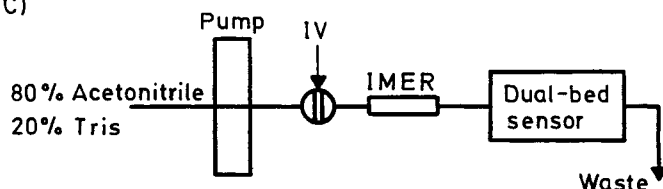


Figure 5: Alternative designs for a sensor containing a packed regenerable reagent and an also recyclable fluorophore. (A) Flow-through sensor system. 1. eluent vessel; 2. pump; 3. injection valve; 4. TCPO reactor; 5. CL cell; 6. light-tight box with PMT; 7. amplifier; 8. recorder. (Reproduced from [40] with permission of the American Chemical Society). (B) Design of the packed two-layer sensor. 1. inlet capillary; 2. inlet cap with frit; 3. quartz tube; 4. TCPO layer; 5. frit; 6. luminophore layer; 7. outlet cap with frit; 8. outlet capillary. (Reproduced from [41] with permission of Elsevier Science Publishers). (C) Flow injection manifold including an immobilized oxidase reactor for the determination of biological analytes. IV injection valve; IMER immobilized enzyme reactor.

Several other sensors based on non-regenerable luminescent reagents potentially compatible with flow systems were used for the determination of oxygen [45], uranyl ion and halides [46]. In the first, oxygen in the sample diffused across a hydrophobic O_2 -permeable membrane into a reservoir containing tetrakis(dimethylaminoethylene) (TMAE), with which it reacted to yield the chemiluminescence, which was measured by means of a light sensor. The sensors for uranyl ion and halides differed from the oxygen sensor in that the former two required the reagent to diffuse in the sample. In using the uranyl sensor, phosphate and nitric acid were diffused into the sample to provide a medium where UO^{2+} would fluoresce. In the halide sensor, Ag-fluorescein was diffused into the sample and halides combined with silver ion to render the reagent (fluorescein) fluorescent.

A cyanide sensor based on the formation of the dicyano-gold complex by reaction between the analyte and the metal gold electrode of a piezoelectric crystal was recently reported [47]. The resulting loss of gold is detected by the piezoelectric crystal as change in the resonance frequency. The system was configured into a flow injection array for ready adaptation to automation. The detection system enables rapid cyanide determinations with little sample preparation or instrument supervision. However, the detection limits obtained (a few micrograms per millilitre) make it unsuitable for real samples. Anions forming soluble or insoluble complexes with gold give rise to over- and underestimated values, respectively.

6.1.5 *Sensors based on an immobilized regenerable reagent*

Reversible sensors are the most desirable of all. However, an irreversible sensor including a regenerable immobilized reagent approaches the functioning of a reversible sensor; the more readily and rapidly the reagent is recycled—which obviously depends on the type of reagent, analyte and interaction between the two—the greater the similarity. The sensors discussed in this section are of regenerable type except in a few cases (*e.g.* pH sensors using acid-base indicators, which are actually reversible devices). Except for a few, which use piezoelectric crystals, all are based on optical techniques. Broadly speaking, the mediator-modified electrodes discussed in Section 1.1 also belong here inasmuch as they include a regenerable reagent (mediator) immobilized on the sensor surface or in the conductive matrix which undergoes a redox process whereby the analyte electrode process is facilitated. However, because this phenomenon can be considered an instance of catalysis, we chose to include such sensors in the previous section.

The demand for measurements of oxygen in gases and liquids at the industrial and biochemical-clinical level has fostered the development of a wide variety of sensors based on fibre optics and different chemical principles, most of which exploit the quenching features of the analyte. The earliest such sensor, reported by Bergman in 1968 [48], used fluoranthene [a strongly fluorescent polycyclic aromatic hydrocarbon (PAH)] adsorbed on a porous glass support, and excitation with a UV light source. The resulting fluorescence is strongly quenched by oxygen and was measured with a photocell. The fluorescence of a variety of other PAHs is known to be quenched by molecular oxygen [49].

Wolfbeis *et al.* developed a variety of (bio)chemical sensor designs based on fibre optics and quenching phenomena. The earliest flow-through sensor of this type for halides and *pseudo*-halides relied on dynamic fluorescence quenching of acridinium and quinolinium indicators, which were immobilized via spacer groups onto a glass surface. The sensors provide the concentrations of dissolved halides via the decrease in the fluorescence intensity due to quenching. The sensitivity to different halides can to some extent be varied by the choice of indicator and increases from chloride to iodide. The detection limits achieved were 0.15 mmol.l^{-1} for iodide, 0.40 mmol.l^{-1} for bromide and 10.0 mmol.l^{-1} for chloride. The reversibility of the sensor response was investigated by pumping halide solutions through the sensing unit until the signal was constant, and then switching to water or a 0.1 mol.l^{-1} sodium sulphate solution. The average response time for a signal change to be indicated by 95% of its signal was *ca.* 40 s [50].

6.2 Flow-through chemical sensors based on integrated separation and detection

One of the most frequent ways of approaching systems in which separation and detection take place sequentially in space and time to current trends in science and technology (*e.g.* automation and miniaturization) is by integrating the two steps. Integrated systems of this type meet the requirements for chemical sensors [14,51–54] and are clearly different from conventional flow systems where detection and mass transfer take place at different locations along the continuous configuration. The characteristic mass transfer of separation techniques occurs simultaneously in this type of sensor. Also, unlike sensors based on integrated reaction and detection (discussed in Section 1), where the reaction kinetics dictates the sensor requirements as regards sensitivity and responsiveness, the sensors described in this section are conditioned by mass transfer.

Integrated separation–detection systems conforming to the definition of sensor may involve three types of interface: gas–liquid (gas diffusion), liquid–liquid (dialysis, extraction) and liquid–solid (any process by which the analyte or one of its reaction products can interact with a solid support, *i.e.* sorption, ion exchange, *etc.*). Other separation techniques involving these types of interface do not allow the development of systems complying with the definition of sensor. For example, the concentration and detection steps in stripping techniques coincide in space, but not in time —concentration always precedes detection—, so electrochemical stripping devices can never be considered sensors. On the other hand, some liquid–liquid extraction systems without phase separation involve continuously monitoring the extracting phase and the kinetics of mass transfer between the two liquids as a result [53–57]. These systems are too complex to be considered sensors. Finally, those systems involving simultaneous gas diffusion and atomic spectroscopic detection (*e.g.* mercury vapour formation or hydride generation and their diffusion across a suitable membrane placed in the sensing zone [58–61]) also fail to meet one of the generic requirements for compliance with the definition of sensor, *viz.* miniaturizability.

The above exclusions leave rather a limited scope for sensors integrating separation and detection, especially with gas-liquid or liquid-liquid interfaces because the gas or ion (molecule) that crosses the membrane gives a signal at the detector. Applications involving liquid-solid interactions are more common since they can be implemented by retaining not only the analyte (one of which intrinsic properties is monitored), but also the product of a prior chemical reaction, which significantly widens the scope of application.

This section is divided into five subsections. The first three are devoted to single-parameter sensors, which are dealt with in three groups according to the type of interface involved: gas-liquid, liquid-liquid and liquid-solid (the most commonplace in this order). The fourth subsection is concerned with multi-parameter sensors, unfortunately much more uncommon. The fifth, final section deals with ion-selective electrodes and those based on field-effect transistors. These last sensors do not integrate separation proper and detection, but somehow involve mass transfer across a membrane or retention, respectively; because they are also frequently applied to environmental samples, they warrant inclusion here. However difficult to classify, excluding them from a chapter on flow-through sensors would have been unjustifiable.

6.2.1 *Flow-through sensors based on integrated gas-diffusion and detection*

Exclusion of gas-diffusion systems integrated with atomic detectors [58–61] for the above-mentioned reasons leaves very few sensors that rely on integrated diffusion and detection because gaseous analytes that lend themselves to direct detection are very scarce and gas-selective electrodes are dealt with in subsection 5. Two such sensors are described below.

One of them is based on semiconductor technology, which allows one to develop small, inexpensive and reliable sensors that can directly be integrated with control and data-handling electronic circuits. The sensor has an ammonia-sensitive metal oxide semiconductor (MOS) design [62,63] and a thin plate of catalytic metal such as iridium as a part of the gate. Iridium-metal oxide semiconductor (Ir-MOS) capacitors are highly sensitive to gaseous ammonia (the detection limit in air is *ca.* 1 ppm/vol or 0.59 mg.kg⁻¹) and can be manufactured with rather good reproducibility. Figure 6.A depicts one such probe. The IR-MOS capacitor (5 × 10 mm²) is placed on a 2 × 7 × 20 mm³ aluminium plate with a ground niche to ensure correct sensor positioning. The aluminium plate is thermostated at 35°C by means of a temperature-sensing diode and a heating resistor placed on the backside of the aluminium plate. The circular flow-through cell (5 mm diameter, 0.2 mm deep) is positioned at the edge. The cavity is connected to an inlet and an outlet that are placed diametrically to let buffer pass through. A gas-permeable membrane (PTFE, pore size 1 μm, diameter 5 mm) and a Teflon gasket (3 mm ID, 5 mm OD, 0.1 mm thickness) are mounted over the cavity. The flow-through cell is pressed against the active surface of the sensor by a spring to ensure tight sealing, mounted on a 5-pin integrated circuit socket and electrically connected to a C-V meter and a temperature control circuit. Ammonia molecules in equilibrium with ammonium ions in a solutions diffuse across the gas-permeable membrane and are detected by the sensor. This phase-separation stage has the advantage that the sensor is thereby electrically insulated from the solution. Background noise is thus decreased,

so use of a reference electrode is unnecessary. This ammonia-N probe (ammonia-N denotes the sum of ammonia molecules and ammonium ions in the solution) has proved to be useful for the determination of the analyte in various biological and non-biological solutions such as whole blood, blood serum, rainwater and river water [64]. The probe was used in combination with an immobilized urease reactor to develop a method for the determination of urea and a flow-injection method for quantitation of creatinine in biological fluids using creatinine iminohydrolase (CIH) immobilized onto CPG in a reactor located prior to the detection system [64]. The amount of ammonia released in the enzyme-catalysed reaction is a measure of the creatinine concentration. The normal background level of ammonia-N in biological samples such as whole blood or urine is roughly similar to or higher than that of creatinine. Consequently, endogenous ammonia-N in a sample has to be removed prior to the determination of creatinine, which is accomplished by co-immobilizing glutamate dehydrogenase with CIH. In this way, endogenous ammonia-N is simultaneously removed, which avoids the need for blank correction.

Trojanek and Bruckenstein developed a flow-through pneumato-amperometric sensor for the determination of nitrite based on a gas-permeable Gore-Tex membrane, one face of which is covered with a porous gold electrode. The flow-cell (Figure 6.B) consists of two Plexiglas parts held together by three screws. The upper part serves as the electrochemical cell and contains 0.1 M sulphuric acid, a gold wire auxiliary electrode, and a capillary leading to a saturated calomel reference electrode. Electrolyte contact to the porous gold working electrode is made through a 0.1 internal diameter hole drilled in the bottom of the upper part. The metallized gold membrane is sealed to the cell bottom around the hole using two layers of pressure-sensitive silicone adhesive through which a 0.1 internal hole is punched. This assembly provides a well-defined working electrode area in contact with the sulphuric acid electrolyte without any leakage of electrolyte from the cell. The bottom part of the detector is screwed tightly to the upper half of the detector, thereby clamping together the metallized membrane, the auxiliary membrane, and the 0.015 internal thick polyethylene spacer cut out to connect the liquid inlet and outlet both of which are attached to the bottom half of the cell. The most severe shortcoming of earlier pneumato-amperometric sensors [65,66] was the long time required for the monitored species to pass from the liquid to the gas phase, which greatly contributed to sluggish determinations. This shortcoming was circumvented by using the set-up depicted in Figure 6.C. X is reacted with R to form Y, and the solution containing X, R and Y is circulated over the gas porous electrode surface. The unmetallized Teflon face of the membrane contacts the aqueous liquid phase containing R, any unreacted X, and Y. This last, which is a volatile electroactive species, partitions into and through the gas pores in the membrane and reaches its metallized face. The potential of the porous metal electrode is selected to be in the limiting current region for electrolysis of Y. Hence, the electrolysis current is proportional to the amount of X from which Y is produced. It should be noted that, even if R is electroactive, it will not yield a current provided it is non-volatile. The response time of this sensor is sufficiently fast to permit flow-injection determinations at a rate as high 100 samples per h.

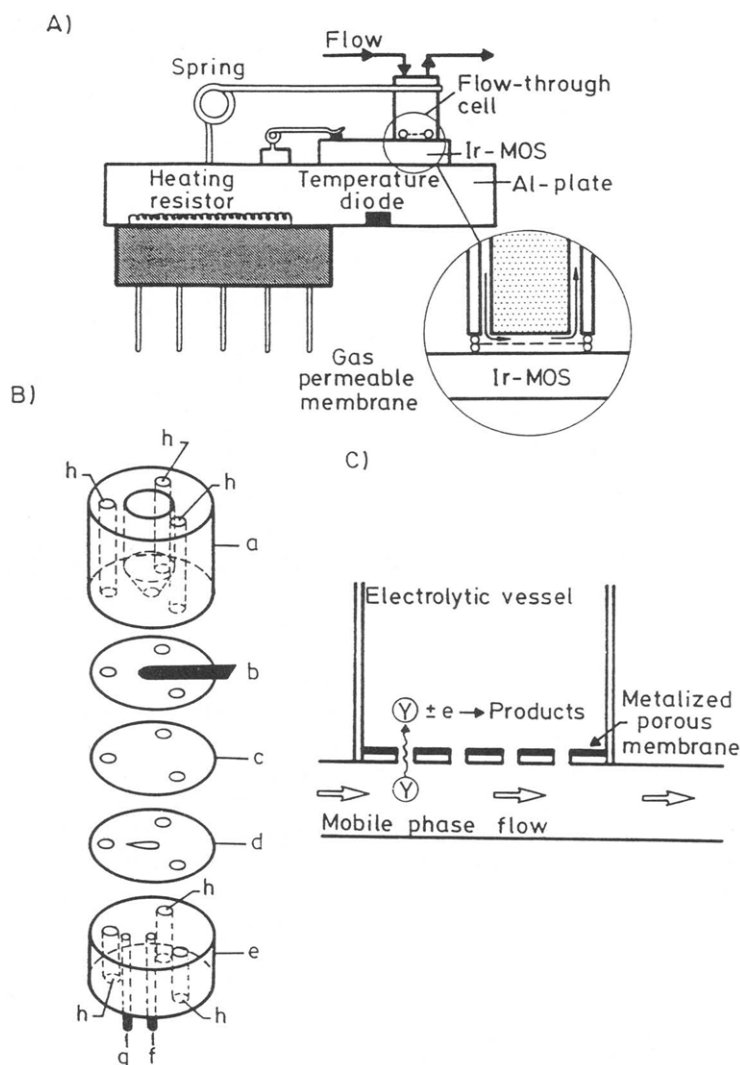


Figure 6: (A) Schematic diagram of the ammonia-N-sensitive probe based on an Ir-MOS capacitor. (Reproduced from [64] with permission of Elsevier Science Publishers). (B) Pneumato-amperometric flow-through cell: (a) upper Plexiglas part; (b) metallized Gor-Tec membrane; (c) auxiliary Gore-Tec membrane; (d) polyethylene spacer; (e) bottom Plexiglas part; (f) carrier stream inlet; (g) carrier stream outlet. (C) Schematic representation of the pneumato-amperometric process. The volatile species Y in the carrier stream diffuses through the membrane pores to the porous electrode surface in the electrochemical cell and is oxidized or reduced. (from [64] with permission of the ACS).

6.2.2 *Flow-through sensors based on integrated liquid-liquid separation and detection*

Flow-through sensors based on integrated optical detection and a liquid-liquid separation are relatively scant since the analytes are rarely determined by their photometric or luminescence properties. Thus, with few exceptions, these sensors use amperometric detection —as noted earlier, ISEs and ISFETs are dealt with in a separate section.

These sensors use two chief types of separation techniques involving a liquid-liquid interface, *viz.* dialysis and liquid-liquid extraction —the latter, despite its potential for future developments, has only been used with a single sensor.

A number of membrane-coated voltammetric electrodes are based on integrated dialysis and detection. The inherent selectivity achieved by having the analyte pass through a membrane of a given cut-off, which allows the species of interest to be isolated from at least those of sizes exceeding the membrane pore size, is furthered by the use of permselective membranes as electrode coats on account of their special properties.

Sittampalan and Wilson studied the effect of coating platinum electrodes with cellulose films in comparison with a bare platinum electrode and arrived at the following conclusions: (a) the current response of the platinum electrode to hydrogen peroxide was virtually unchanged by exposure to complex environmental samples, as reflected in the identical peak currents obtained —the typical poisoning effect of these matrices was thus seemingly overcome; and (b) injections of blank complex matrices gave rise to negligible currents, thus indicating that virtually no electroactive species diffused across the film [67].

One other design developed by Wang's group uses the same base sensor (GCE), which is coated with a layer of poly(4-vinylpyridine) (PVP). This cationic polyelectrolyte was one of the first polymers used to modify electrode surfaces. Much research effort in this context has been directed to the characterization of the transport and electrostatic binding of multi-charged anions at PVP-coated electrodes. The ability of this polymer to bind counterionic reactants has been exploited for preconcentration of analytes from dilute solutions. Lowering the overvoltage for some analytes via electroactive reagents immobilized in PVP is one major analytical advantage of PVP-modified electrodes. Based on this evidence, Wang *et al.* built a sensor of this type for use in hydrodynamic systems (HPLC and FIA) by using a straightforward procedure involving immersion of the bare CGE for 30 s in a stirred solution containing 0.4 % polymer in ethanol following polishing with albumin and rinsing with bidistilled water. The coat film remaining after the solvent was evaporated was 11 μm thick. The most substantial effect of the protonated PVP film is the enhanced selectivity arising from exclusion of cationic species from the surface. Notwithstanding the increased diffusional resistance resulting from the presence of the membrane, detection limits as low as *ca.* 0.04 ng ascorbic acid and 0.10 ng uric acid were obtained. Protection from organic surfactants was coupled to the charge-exclusion effect by using a bilayer coating in the form of a cellulose acetate film atop the PVP layer (deposited by syringing 10 μl of 5 % cellulose acetate solution in 1:1 acetone/cyclohexanone on top of the PVP layer, followed by 40-min hydrolysis in a stirred 0.07 mol.l⁻¹ KOH solution). In this way, the sensor performed brilliantly in the determination of the analytes in urine samples [68].

Integrating liquid–liquid extraction and detection is far from easy, as reflected in the few attempts made so far. Many of the devices developed for this purpose fail to comply with the definition of a sensor. Such is the case with the continuous liquid–liquid extraction systems without phase separation. By programmed switching of the propulsion system (a peristaltic pump), the extracting phase is passed iteratively by the detection point in a to-and-fro motion that enriches it gradually with the extracted species [55–57]. The system is too complex to be considered a sensor, though; in addition, the extraction process is not completely simultaneous with detection. The device proposed by Pawliszyn, based on the establishment of a concentration gradient, complies more rigorously with the definition of sensor. However, it can only be used for detection techniques capable of probing the typically small volumes associated with diffusion layers, which are most often lower than 100 μm . Figure 7 shows the two designs proposed by Pawliszyn. One of them (Figure 7.A) consists of a cellulose nitrate membrane of 0.2 μm pore size that is glued carefully onto one end of a piece of 2 mm OD \times ca. 1 mm ID glass tubing. The capillary is inserted into a 5-cm length of 3-mm ID tubing and aqueous samples introduced into the system via a 0.5-mm OD fused silica capillary. The pure solvent (water) is delivered by means of microtubing made of polyfluorocarbon resin. The other design (Figure 7.B) consists of a single silicone hollow fibre membrane of 0.3 mm ID and ca. 0.6 mm OD placed in a 2-cm long \times 1 mm ID piece of square tubing. The stripping fluid (hexane or water) flows through the centre of the fibre while the aqueous samples flow around the outside of the fibre and enter the system via a fused silica capillary. The diffusion modules are attached to a vertical translation stage to allow precise focusing of the laser beam onto the membrane surface. The concentration gradients formed during the extraction of analytes from an aqueous to an organic phase can be quantified via the refractive index gradient by measuring the deflection of the focused laser beam that passes near the interface between the two phases. The maximum of the concentration gradient transient is proportional to the analyte concentration in the sample. The steep concentration gradients created at the interface during the initial stages of the mass transport process ensure good sensitivity. The detection limit of this technique is proportional to the distance of the probing laser beam from the interface and the diffusion coefficient of the analyte in the organic phase. The sensitivity of this special type of sensor is related to other properties of the organic phase as described by the distribution constant. In addition, separation of species with substantially different diffusion coefficients is fairly easy to accomplish since the time corresponding to the maximum of the transient is inversely proportional to the diffusion coefficient. The sensor enables very rapid analytical determinations since quantitation is performed at the initial stages of sample preparation [69].

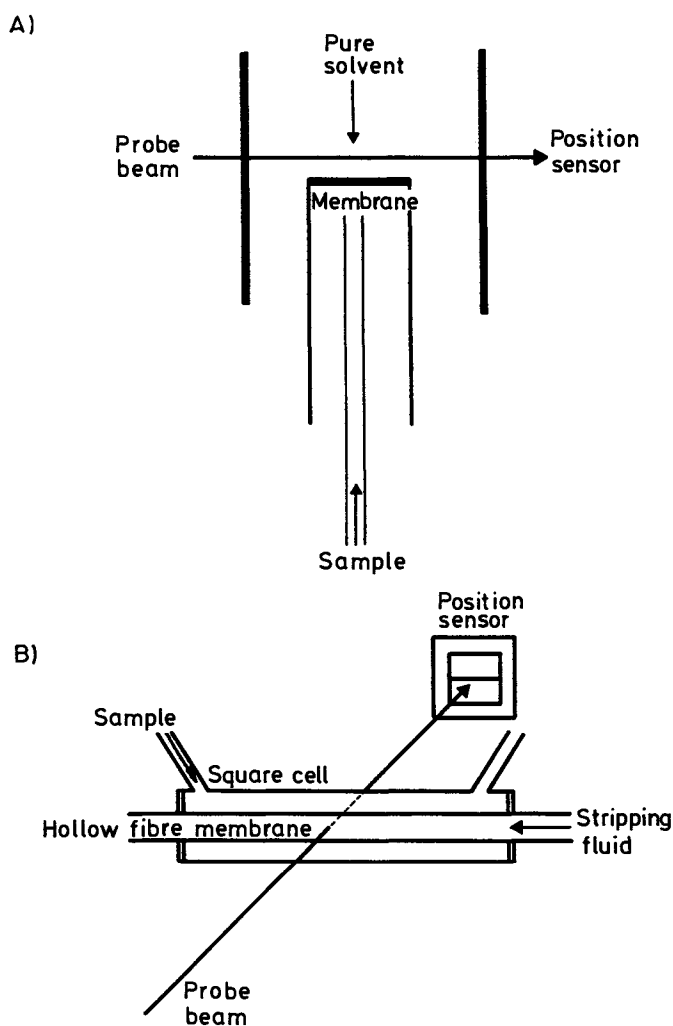


Figure 7: Experimental designs using a planar porous membrane (A, top) and a silicone hollow fibre membrane (B, bottom) for the implementation of sensors based on integrated liquid-liquid extraction and detection. (Reproduced from [69] with permission of the American Chemical Society).

6.2.3 *Flow-through sensors based on integrated sorption and detection*

Most flow-through sensors integrating retention and detection involve placement of an inert support in the flow-cell of a non-destructive spectroscopic detector where the analytes or their retention products are retained temporarily for sensing, after which they are eluted. Rendering these sensors reusable entails including a regeneration step suited to the way the retention is performed. In the best of cases, the carrier itself acts as regenerator; otherwise, a continuous configuration (usually an FI manifold) is the most convenient choice for this purpose. The equipment required to develop this type of sensors is very simple and resembles closely that used to implement ordinary liquid–solid separations in FI manifolds. The only difference lies in the replacement of the packed reactor located in the transport-reaction zone with a packed —usually photometric or fluorimetric— flow-cell accommodated in the detector. Whether the packing material is inert or active, it should meet the following requirements: (a) its particle diameter should be large enough ($< 80\text{--}100\ \mu\text{m}$) in order to avoid overpressure; (b) it should be made of a material compatible with the nature of the integrated detection system (e.g. almost transparent for absorbance measurements); and, (c) the retention/elution process should be fast enough to avoid kinetic problems. Although somewhat specialized, integrated flow-cells are normally commercially available. Ideally, they should be short (0.2–1.5 mm) and narrow-bore in order to avoid problems arising from inadequate detector capacity and sensitivity, respectively. Ideally, they should also have small inner volumes in order to boost sensitivity and sample throughput.

Luminescence sensors are fairly commonplace in environmental analysis. Thus, the configuration depicted in Figure 8.A was used by Cañizares *et al.* to implement a sensor for aluminium based on the formation of a fluorescent complex between the analyte and salicylaldehyde picolinoylhydrazone. The sensor was built from a commercially available flow-cell for fluorimetric measurements (Hellma 178.12QS, 1.5 mm pathlength) that was packed with C_{18} bonded silica beads and placed in an ordinary fluorimeter. The complex, formed along the manifold on merging the injected sample with a reagent stream, was retained in the sensor, measured, and finally eluted with $200\ \mu\text{l}$ of $2\ \text{mol.l}^{-1}\ \text{HCl}$ injected near the detector. The method thus implemented features a linear determination range from 2 to $200\ \text{ng Al/ml}$, good repeatability and excellent selectivity [70]. However, the ideal continuous configuration for implementation of sensors based on retention of the product of a prior reaction is one of symmetric merging zones (Figure 8.B), with or without a switching or injection valve after the sample and reagent insertion valve. The need for one such auxiliary valve is dictated by whether or not an eluent other than the carrier is required. This type of configuration and fluorescent complex-formation reaction has been used to implement various sensors for anions.

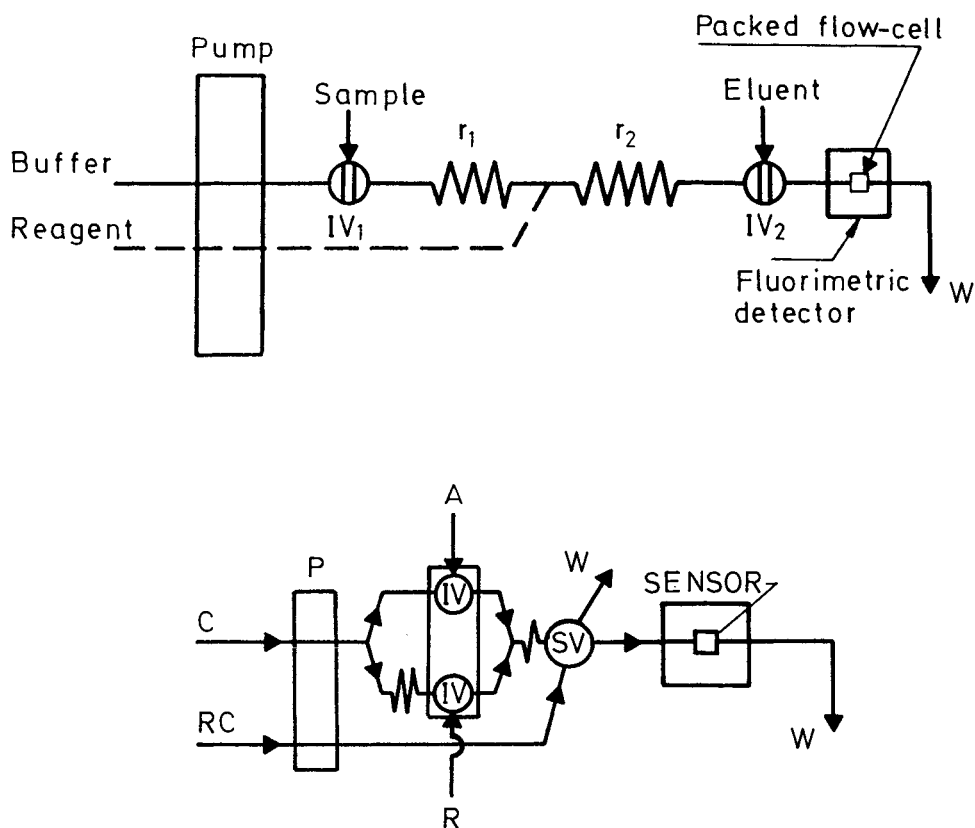


Figure 8: General manifolds for implementation of methods using optical sensors. (A) Direct measurement of the intrinsic features of the analyte (single-channel system) or a reaction product (the reagent delivery channel, represented by the dotted line, is included). (B) Symmetric merging zones configuration for measurement of reaction product. R reactor; S sample; C carrier; RC reagent carrier; IV injection valve; SV switching valve; R reagent; W waste.

The first such sensor, developed for the determination of fluoride ion, relies on the formation of the zirconium(IV)–Calcein Blue–fluoride complex and its retention in an ionic resin (DEAE–Sephadex) packed in a commercially available flow-cell such as that shown in Figure 9.A (a Hellma 178.12QS model) [71]. The sample and the zirconium–Calcein Blue binary complex are simultaneously injected into a water and a $5 \times 10^{-3} \text{ mol.l}^{-1}$ HCl stream, respectively, which form the fluorescent species on merging prior to the detector; there, the ternary complex is retained for the time needed to acquire the analytical signal and subsequently eluted by injecting $850 \mu\text{l}$ of 0.4 mol.l^{-1} HCl via a third valve located near the detector. This sensor exemplifies the advantages of flow-through sensors over conventional, probe-type sensors. Table 1 compares the typical parameters for the flow-through sensor and an earlier probe sensor based on the same chemical reaction. As can be seen, the former clearly surpasses the latter in terms of sensitivity, reproducibility, service life and sample throughput. Also, the selectivity of this sensor is much higher than that of the manual method based on the same reaction [73].

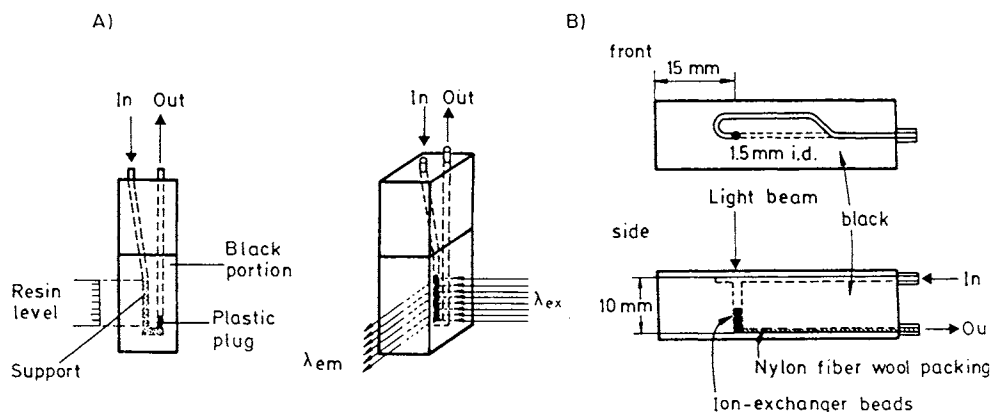


Figure 9: Flow-through cells for spectrofluorimetric sensors. (a) Flow-through cell: a fused silica tube (1.5 mm ID), packed with 1 mg of CM-Sephadex C-25. (Reproduced from [72] with permission of Elsevier Science Publishers); (b) Micro-cell holder. (Reproduced from [76] with permission of the Royal Society of Chemistry).

Table 1: Comparison of the performance of a probe-type and a flow-cell type sensor for fluoride. Reproduced from [71] with permission of Elsevier Science Publishers

Parameter	Probe-type sensor	Flow-cell type sensor
Determination range	0.5-8 $\mu\text{g}.\text{ml}^{-1}$	1-40 $\text{ng}.\text{ml}^{-1}$
Detection limit	0.5 $\mu\text{g}.\text{ml}^{-1}$	1 $\text{ng}.\text{ml}^{-1}$
Time for:		
Preparation	overnight	< 15 min
Measurement step	30 min	1 min
Regeneration step	60 min	1 min
Reproducibility	15 %	1 %
Sensor lifetime	15 measurements	> 100 measurements

The cyanide sensor developed by the authors' group is based on the reaction of cyanide ion with pyridoxal-5-phosphate and the subsequent retention of the product formed in the sensor (a fluorimetric flow-cell packed with QAE-Sephadex resin). The eluent is not injected, but merged with a stream of 0.05 $\text{mol}.\text{l}^{-1}$ HCl after the reactor which is intended to both acidify the complex and elute it after measurement. The calibration graph for the target analyte was linear from 50 $\text{ng}.\text{ml}^{-1}$ to 3.0 $\mu\text{g}.\text{ml}^{-1}$, and the relative standard deviation and sample throughput were 1.4% (for 2 $\mu\text{g} \text{CN}^{-}.\text{ml}^{-1}$) and 10 samples per h, respectively. The selectivity of this sensor is clearly higher than that of conventional continuous methods, both fluorimetric and photometric [74].

Solid-phase absorptiometry, developed in depth by Yoshimura *et al.* [75], is the most immediate precursor of the sensors discussed next. One of the few existing flow-through sensors based on direct measurements of the analyte absorption was developed by this Japanese group for the determination of copper [76]. They used a single-channel manifold including two serially arranged injection valves for the sample and a regenerating nitric acid solution in addition to a conventional photometer. The sensor consisted of a commercially available flow-cell (Figure 9.B) that was packed to an appropriate height with Bio-Rad AG50W-X12 resin of 100–200 mesh in hydrogen form. The photometer was placed vertically in order to make the top part of the ion-exchange material flat. The calibration graph run was linear over the range 0.01–0.5 μmol copper.

An integrated sensor for the determination of formaldehyde based on retention of the reaction product of the analyte with *p*-rosaniline and sulfite in a flow-cell packed with Dowex 1-X-8 anion resin was developed by our group [77]. The complex manifold required for this purpose is depicted in Figure 10. The sample (1 or 2 ml, depending on the analyte concentration) was inserted into a bidistilled water stream that was subsequently merged with a stream of *p*-rosaniline/HCl/Na₂SO₃ at M₁. The reaction product formed in reactor R started to be retained on the resin 55 s after injection as the switching valve (SV) was turned to position 1. The signal exhibited by the retained product was monitored at 560 nm. When the signal reached its maximum height (*i.e.* on passage of the sample plug tail), the concentrated reaction product was eluted with 2 mol.l⁻¹ HCl saturated with 1-butanol, which was inserted via position 2 of SV 80 s after injection in order to restore the baseline. The method thus developed features good selectivity, a detection limit of 0.3 μg.ml⁻¹ (1 ml sample) or 75 ng.ml⁻¹ (2 ml sample), and a linear determination range between 1 and 30 μg.ml⁻¹. The *rsd* obtained was 2.8 % and 1.3 % for 2 and 20 μg formaldehyde/ml, respectively. The sampling frequency achieved was 10 h⁻¹. The method was applied to the determination of formaldehyde in well water.

The response mechanism of the conducting polymer poly(pyrrole) to a selection of gases and vapours was investigated by Slater *et al.* by using two techniques based on measurements of resistance and mass changes by means of piezoelectric quartz crystal microbalances in order to characterize responses for incorporation in sensor arrays. For this purpose, bromide-doped films were exposed to methanol, hexane, 2-2-dimethylbutane, ammonia and hydrogen sulphide. Polymer sheets of variable thickness were also exposed to methanol in order to study their responses. This last approach was seemingly the most promising. Methanol absorption into poly(pyrrole) appears to be a two stage process involving firstly the penetration of vapour into the polymer, accompanied by swelling and then diffusion at an increased rate into the swollen rubbery material. Full recovery was not attained after the first exposure, leading to a second exposure with a modified polymer containing initial concentrations of methanol. As swelling is the rate-determining step of the sorption, subsequent exposures take the polymer to the same point in the sorption curves as the first 5 min exposure. A systematic study involving various gasses and vapours revealed that the response mechanism of (poly/pyrrole) sensing was due to a mixed response involving electronic and physical effects. Further developments in mass sensors for gases based on the use of these materials call for further research [78]. In this context, Krawczynski *et al.* studied various crystal-coating materials such as the hydrochlorides of pyridoxine, glutamic acid, histidine, methionine, alanine and cysteine with a view to constructing ammonia sensors. The first three coating materials enabled detection of the analyte at concentrations between 1 ng/ml and 1000 μg.ml⁻¹, and provided sensors with service lives of 1–4 months. Other coating materials tested for the determination of SO₂ provided very promising results.

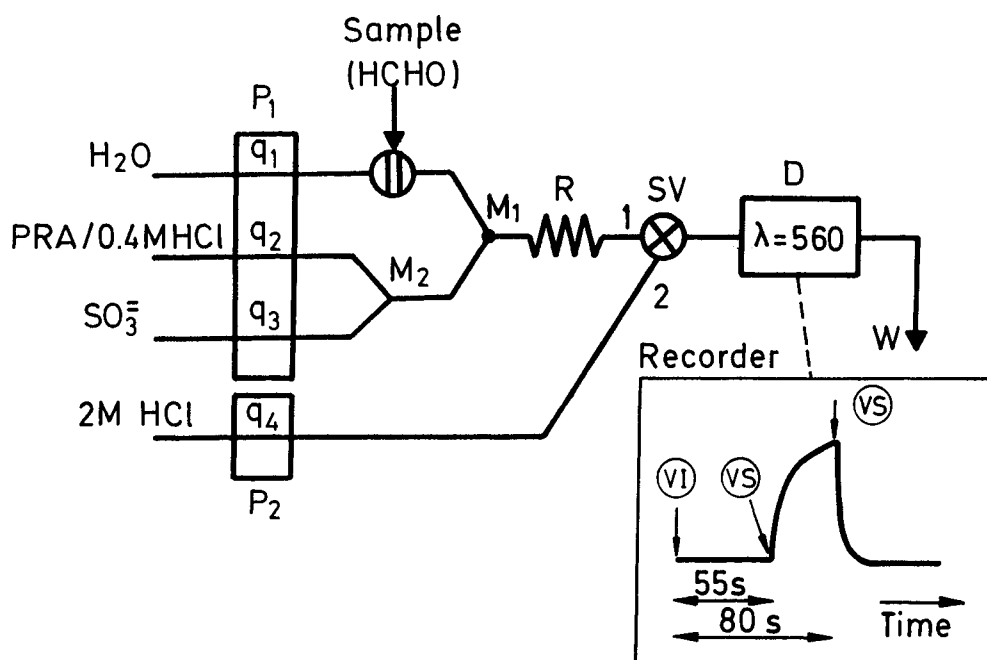


Figure 10: Manifold used and recording obtained with the flow-through photometric sensor for the determination of formaldehyde. PRA *p*-rosaniline, *q* flow-rate, *P* peristaltic pump, *M* mixing point, *R* reactor, *SV* and *IV* switching and injection valve, respectively, *D* detector and *W* waste. (Reproduced from [77] with permission of Marcel Dekker).

6.2.4 *Flow-through sensors for multideterminations based on integrated retention and detection*

Sensors allowing two or more analytes to be determined by a simple procedure are technically more complex than single-parameter sensors, but also more advantageous, which justifies the R&D endeavours devoted to them. Few multi-parameter sensors have so far been developed, however, and even fewer are actually operative, among which those commercially available are numbered. Progress in this area is therefore much needed and desired. Multi-determinations based on sensors can be accomplished by (a) using a detector capable of distinguishing the signal produced by each analyte, whether as such or on subjection to a chemical reaction; and (b) by separating the analytes using an on-line coupled chromatographic or non-chromatographic technique, aided or not by a prior chemical reaction. Depending on the particular approach used, multi-determinations can be simultaneous (which, in FIA terms, means that two or more analytes are determined in a single injection operation [80]), or sequential (each analyte requires one injection).

The ability of diode array spectrophotometers to monitor in a simultaneous manner the absorbance at several wavelengths allows one to select specific λ values at which the behaviour of the analytes (or their reaction products) is sufficiently different for discrimination purposes. Thus, the authors' group developed a method for the resolution of mixtures of amines (2,4-dinitrophenylhydrazine, 2-nitrophenylhydrazine and 4-nitrophenylhydrazine) based on (a) the use of a single-channel hydrodynamic system and (b) a flow-cell packed with a suitable material (C_{18} resin); (c) placement of the flow-cell in a diode array spectrophotometer; and (d) acquisition and processing of the intrinsic absorbance data for the analytes at the selected measuring wavelengths. Figure 11 shows the absorption spectra of the target analytes; as can be seen, they were extensively overlapped, so they could only be distinguished by careful selection of the wavelengths at which the individual spectra were most markedly different from one another. The experimental procedure was as follows: the sample containing the analytes was injected to a stream of 6:4 v/v phosphate/methanol buffer acting as carrier and eluent. Because no chemical reaction was needed prior to detection, the distance between the injection and detection points was minimal, which resulted in a short residence time. The fast kinetics of retention/elution allowed a determination limit of $5 \times 10^{-7} \text{ mol.l}^{-1}$ to be achieved. By solving the 10-equation system obtained from measurements made at 10 different wavelengths, mixtures of these amines were resolved with fairly small errors [81].

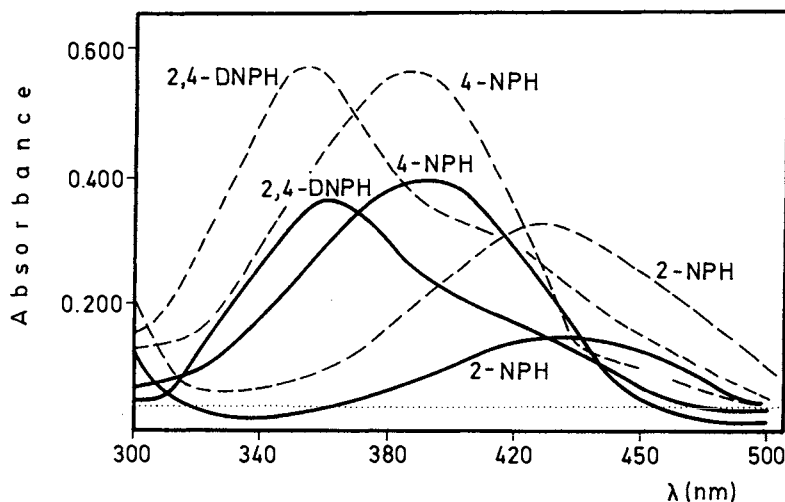


Figure 11: Absorption spectra of 2,4-dinitrophenylhydrazine (2,4-DNPH), 4-nitrophenylhydrazine (4-NPH) and 2-nitrophenylhydrazine (2-NPH) in solution (solid line) and retained on C_{18} bonded silica of 60-100 μm particle size (broken line). The dotted line corresponds to the blank spectrum in the second type of experiment (cell packed with C_{18} bonded silica in distilled water). (Reproduced from [81] with permission of Elsevier Science Publishers).

A photometric flow-through sensor for the determination of carbamate pesticides (carbofuran, propoxur and carbaryl) based on similar principles as regards the detector and sensor used (a diode array spectrophotometer and a flow-cell packed with C_{18} resin) was used to monitor the formation of the products resulting from hydrolysis of the analytes and on-line coupling of the respective phenols with diazotized sulphanilic acid. This preliminary reaction was implemented in an FI manifold such as that depicted in Figure 12, where the sample was injected into an aqueous carrier and merged with a basic stream to hydrolyse the analytes along reactor R_1 . On mixing of the nitrite and sulphanilic acid streams, the diazotation reaction took place in R_2 , by the dye formation on merging with R_1 prior to the flow-cell, where the dye was retained and detected. After absorbance-data pairs were acquired at the nine selected wavelengths, actuating valve SV drove a stream of 1:1 ethanol/2 mol.l⁻¹ HNO₃ to the sensor, from which the retained products were rapidly flushed. The sample throughput thus achieved was 40 samples/h and the determination range encompassed concentrations between a few nanograms and a few micrograms per millilitre. The sensor was used to resolve mixtures of the target analytes in various types of water with excellent results. The enhanced sensitivity arising from *in situ* concentration was apparent in a comparison of the determination limits obtained with those afforded by a conventional FIA method based on the same continuous configuration but including no packing in the flow-cell (the former were up to 50 times lower than the latter) [82].

A prior separation can be used to facilitate the sequential arrival of several species at the sensor and hence enable their temporal discrimination. Such discrimination can be accomplished in two ways, namely (a) by removing each analyte or product before the next arrives (the detector response corresponds to a single species in each case), and (b) by allowing the analytes or products to accumulate, which gives rise to a step-line response, and removing them as a whole after measurement. The species to be measured in both cases are similar and exhibit signals at the same instrumental settings. Multi-parameter measurements in turn can be either simultaneous or sequential. The former are performed in a single injection. This operational mode usually calls for a high resolving power such (e.g. that of a chromatograph). On the other hand, sequential measurements require individual injections (one per analyte).

Tena *et al.* used an HPLC/post-column derivatizing-integrated reaction/detection sensor system for the determination of carbaryl and its hydrolysis product based on separation of the two analytes with the aid of an ultrabasic C_{18} chromatographic column and derivatization prior to retention in the sensor, in addition to formation of a dye with sulphanilic acid (previously diazotized on-line), which followed hydrolysis of carbaryl. After measurement, the products were eluted by using a stream of 1:1 v/v ethanol/2 mol.l⁻¹ HNO₃ that was driven to the detector by actuating a switching valve near the detector. The method thus developed allows the determination of the two analytes at concentrations between 5 and 800 ng.ml⁻¹, with an rsd less than 4% and excellent selectivity against other carbamate pesticides [53].

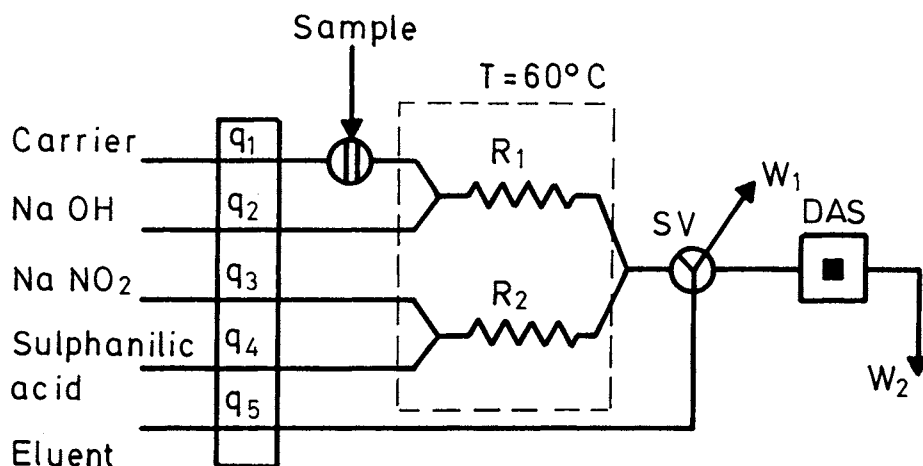


Figure 12: Manifold used for implementation of the flow-through sensor for the determination of carbamate compounds based on hydrolysis of the analytes and dye formation, and recordings obtained by using each manifold. q flow-rate, R reactor, SV switching valve, DAS diode array spectrophotometer, W waste. (Reproduced from [82] with permission of the American Chemical Society).

If the discriminating power of the separation system concerned is inadequate, chemical derivatization is one possible aid. Such is the case with the sensor for speciation of aluminium, which uses the Driscoll method [84] to distinguish the different forms of the metal on the basis of the above-described fluorescent complex-formation with a hydrazone. Figure 13 shows the FI manifold used in conjunction with the sensor. A valve located prior to the injection port allowed switching between two sample pH values in order to determine total reactive aluminium (pH 1.0) or total monomeric aluminium (pH 3.5). Valve IV_2 was kept in its load position in both determinations, so plugs injected via IV_1 did not pass through the exchange column. A third injection with IV_2 in its unload position allowed the sample plug to pass through the column in order to retain charged species and determine non-labile monomeric aluminium. The column was recycled upstream by switching valve IV_2 to its load position. In this way, more efficient regeneration was achieved and gradually increased compactness in the packing material, which usually results in flow-rate oscillations and hence irreproducibility, was avoided. In addition to the three species determined in the three injections, labile monomeric aluminium was quantitated as the difference between total

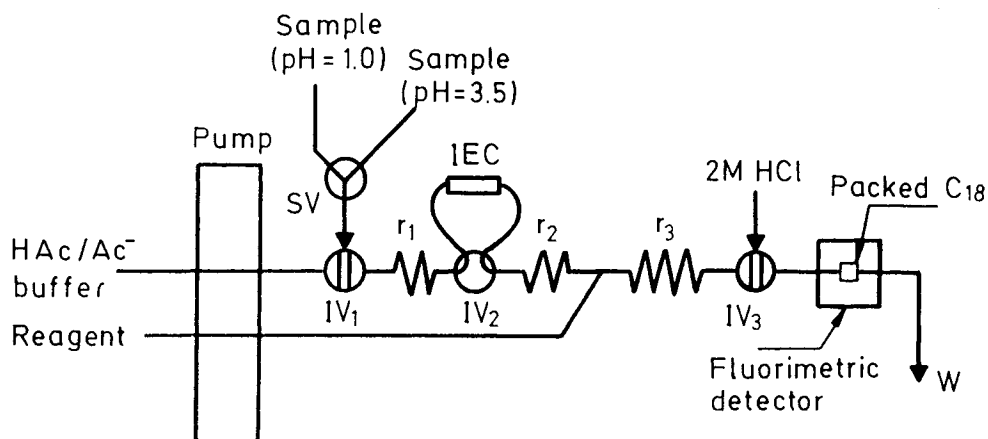


Figure 13: Flow-through sensor for aluminium speciation coupled to an FI manifold for implementation of the Driscoll method. SV switching valve; IV injection valve; IEC ion-exchange column; r reactors; W waste.

monomeric and non-labile monomeric metal. The sensor was regenerated after each determination and thus made ready for the next one by switching injection valve IV₃ to insert 200 μl of 2 mol. l^{-1} HCl [85].

6.2.5 Ion-selective electrodes and ion-sensitive field-effect transistors

Even though the first electrochemical sensors based on potentiometric principles were developed early this century (e.g. the glass electrode for pH measurements was devised by Cremer in 1906 [107]), the most dramatic advances in this field have been reported in the last 20 years and involve both ion-selective electrodes (ISEs) and ion-sensitive field-effect transistors (ISFETs). The advantages derived from their use in hydrodynamic systems (particularly FI systems) were demonstrated by Cammann [86].

Ishibashi *et al.* devised a potentiometric sensor for the determination of non-ionic surfactants which they improved in several steps. Initially, the authors used a sensor based on a PVC membrane plasticized with 2-nitrophenyl octyl ether that was responsive to cationic complexes formed between a dissolved metal ion and non-ionic surfactants in the sample [87]. At a later stage, they studied the effect of foreign species and elucidated the perturbation from ionic surfactants [88], which they eventually overcame by inserting an ion-exchange column into the base system [89].

Flow-cells accommodating ion-selective electrodes have also been used for continuous-flow measurements in air-segmented streams without debubbling. One such cell is depicted in Figure 14.A. The body is machined from a Plexiglas cylinder (2-cm long \times 5 cm ID). Two Teflon sample guides are screwed into the bottom of the cell body, each having a narrow inlet channel. The indicator and reference electrodes are supported in a vertical position by steel springs serving as connecting wires for the mV/pH meter. The electrodes are positioned in such a way that the flow is introduced at the centre of the membrane face and is forced to exit between the membrane face and the top surface of the sample guide around the membrane perimeter into the reservoir. Air bubbles passing quickly between the membrane face and the surface of the sample guide never collect under the membrane covering the whole sensing surface, so the contact between indicator and reference electrodes is never broken. By using a flow-rate of $4 \text{ ml} \cdot \text{min}^{-1}$, throughputs of 450 samples/h can be achieved in chloride and fluoride determinations, respectively, with rsd values better than 2% [90].

The potential of ISEs for multi-determinations in continuous systems has been exploited by several authors in the form of various cell designs. Thus, Cardwell *et al.* developed the flow-cell depicted in Figure 14.B for the determination of four ions simultaneously with detection by using solid-contact polymer-membrane ion-selective electrodes. The flow-cell design was intended to ensure minimum dispersion and void volumes, a high flow velocity, good contact of the bulk sample plug with each membrane, a well-defined, stable flow across all membranes, and efficient use of the sample plug by making the membranes large relative to the total cell area and arranging them serially in the cell. They cast PVC membranes from tetrahydrofuran solutions onto silver wire contacts in one half of the cell. The membrane compositions were recommended formulations, viz. valinomycin for potassium, neutral carrier ETH 1001 for calcium and tetradodecylammonium nitrate for NO_3^- ; on other hand, chloride ion was measured by means of an Ag/AgCl electrode. A FORTH program was used to expedite acquisition of the data required for flow injection measurements. The system utility was demonstrated by applying it to the simultaneous determination of potassium, calcium, nitrate and chloride in soil extracts; the results compared well with those afforded by standard procedures (see Table 2) [91]. The system was combined with two spectrophotometric transducing flow-cells for the simultaneous determination of potassium, calcium, ammonium, chloride, nitrate and phosphate in a number of plant nutrient solutions [92].

Table 2: Results obtained in the determination of four ions in soils by using the sensor of Cadwell *et al.* and various standard methods

Sample number	Concentration found ^(a) (mg/l)							
	Chloride		Nitrate		Potassium		Calcium	
	IC	FIA	IC	FIA	AAS	FIA	AAS	FIA
1	57.6	57.9 (3.2)	177	154 (0.8)	98.3	94.9 (1.3)	56.4	59.2 (0.8)
2	28.8	30.6 (2.4)	254	225 (1.3)	61.0	52.7 (1.0)	61.5	65.0 (2.3)
3	22.2	19.3 (1.8)	144	125 (1.3)	50.1	41.7 (0.3)	40.5	44.1 (1.9)
4	45.9	57.9 (1.8)	911	865 (0.7)	273	278 (0.4)	181	183 (1.0)
5	47.1	47.2 (1.3)	399	388 (1.2)	238	237 (0.8)	114	110 (1.2)
6	101	97.2 (2.3)	644	617 (0.8)	241	244 (0.3)	218	217 (1.0)
7	20.4	20.3 (2.9)	243	213 (0.9)	209	203 (0.3)	173	154 (1.2)

^(a) Values in brackets are the relative standard deviations ($100\sigma/\text{mean}$) for 4–5 determinations (Reproduced from [91] with permission of Elsevier Science Publishers)

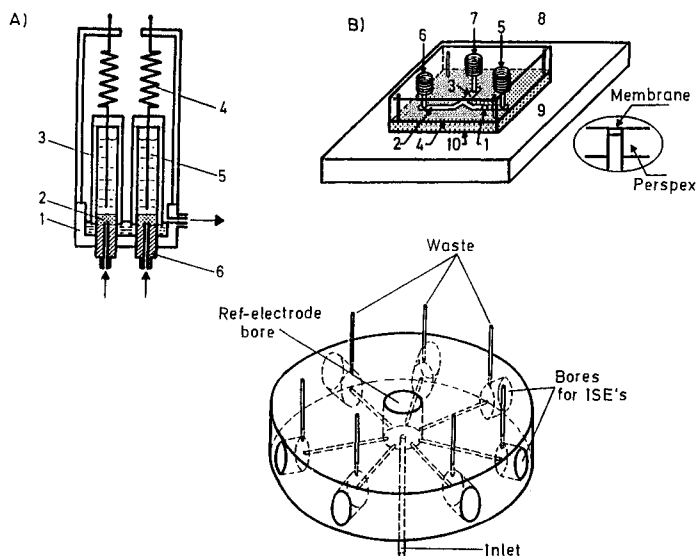


Figure 14: (A) Bubble-through flow cell. (1) Cell body; (2) membrane; (3) ion-selective electrode; (4) steel spring; (5) inner reference solution; (6) sample guide. (Reproduced from [90] with permission of Elsevier Science Publishers). (B) Flow-cell: (1) ion-selective electrodes; (2) reference electrode; (3) Pt wire for grounding; (4) teflon gasket; (5) carrier; (6) inlet for reference solution; (7) waste; (8) screws; (9) diecast box; (10) rubber sheet for sealing. (Reproduced from [91] with permission of Elsevier Science Publishers). (C) Seven-electrode holder: the reference electrode is placed from the top into the central bore. ISEs for Na^+ , K^+ , Ca^{2+} , NO_3^- , Cl^- , and HCO_3^- (NH_3 electrode with internal buffer of $0.1 \text{ mmol.l}^{-1} \text{ NaHCO}_3$) are placed horizontally around the reference electrode; the metal waste tubes are connected to the waste via filter-paper strips. (Reproduced from [86] with permission of VCH Publishers).

A multi-ion drinking water determination of the principal species analysed for in this type of samples (*viz.* sodium, potassium, calcium, bicarbonate, nitrate and chloride) was developed by taking advantage of commercially available ISEs for all these ions. Figure 14.C shows the 7-electrode holder used, which was included in an FI configuration. Because the carrier solution contained 0.1 mM NaHCO_3 , KNO_3 , and CaCl_2 , negative signals were obtained when the actual concentrations of the corresponding ions were low. The expeditiousness of the determination (6 ions.min^{-1}), the small amount of sample needed ($200 \mu\text{l}$) and the low cost of this assembly defy competition. It was extremely useful for routine

screening analysis in this field. Since ISEs have a tendency to detect more in the presence of high concentrations of interfering ions, the screening analysis has a sort of safety device built in. Samples above a certain limit can thus be put aside for analysis by a more elaborate method [86].

The selectivity of ISEs used in flow systems can be boosted by incorporating an on-line separation unit (e.g. an ion-exchange column, a dialysis module or a gas-diffusion device) into the hydrodynamic system. Occasionally, particularly when an ion-exchange column is used, the ancillary unit is employed with a view to improving the sensitivity (through preconcentration) rather than the selectivity.

Okabayasi *et al.* used two identical columns containing Alizarin-Fluorine Blue sulphonate-lanthanum complex in an alternate fashion in order to increase the sample throughput in the preconcentration/separation of fluoride prior to its determination by means of an ISE. By switching valve SV, the analyte from a sample was measured and eluted while that in the next sample was retained in the other column [93].

In the method proposed by van Staden for the determination of three halides, these are separated on a short column packed with a strongly basic ion-exchange resin (Dowex i-X8) that is placed in an FI manifold; also, a laboratory-made tubular silver/silver halide ion-selective electrode is used as a potentiometric sensor. Van Staden compared the response capabilities of the different halide-selective electrodes to a wide concentration range (20–5000 $\mu\text{g}.\text{ml}^{-1}$) of single and mixed halide solutions in the presence and absence of the incorporated ion-exchange column. By careful selection of appropriate concentrations of the potassium nitrate carrier/eluent stream to satisfy the requirements of both the ion-exchange column and the halide-selective electrode, it is possible to separate and determine chloride, bromide and iodide in mixed halide solutions with a detection limit of 5 $\mu\text{g}.\text{ml}^{-1}$ [94].

With regards to ammonia, groundwater treatment plants regularly require a knowledge of the ammonium ion content in the incoming water; this is a variable parameter, particularly in densely populated, highly industrialized hydrographic basins. It is also one of the parameters that must be measured in order to determine the chlorine dose to be added in the treatment process. A method allowing the ammonium ion concentration in the incoming waters to be monitored in a continuous fashion can be used to determine the exact amount of chlorinating agent to be added, thereby saving resources and improving water quality. This is the source of the wide use of ammonium ISEs in continuous systems for this purpose. A specially constructed all-solid state tubular flow-through ammonium electrode was used by Alegret *et al.* in conjunction with a gas-diffusion chamber to enhance its selectivity. The electrode, a modified design of other flow-through tubular PVC matrix membrane electrodes without an inner reference solution [95–97], is shown in Figure 15.A. The conductive support onto which the membrane was applied was an epoxy resin loaded with graphite. The inner diameter of the channel drilled in the conducting support was 1.5 mm and once the sensing membrane layers had been deposited dropwise, the inner diameter of the tubular channel was reduced to ca. 1.2 mm (membrane thickness, ca. 0.15 mm). The FI manifold into which the sensor was incorporated is depicted in Figure 15.B. The sample was inserted into a distilled water stream that was merged with a basic stream in order to assure the presence

of ammonia as such on passage through the gas-diffusion cell. The acceptor stream consisted of Tris buffer which drove the analyte to the detector. Up to 30 samples/h were processed with a detection limit of *ca.* 10^6 mol.l^{-1} . The system was tested with excellent results on a water treatment plant [98]. A similar configuration including a tubular AgI/Ag₂S electrode was employed for the determination of free and weakly complexed cyanide [99].

Ion-selective field-effect transistors (ISFETs) are the offspring from the marriage of ion-selective electrodes and solid-state electronics. They avoid the noise problems of ISEs arising from the high electric impedance of the ion-selective membrane used on bringing it into contact with the amplifier. There are two types of ISFETs: metal-insulator capacitors and transistors. The semiconductor used is normally silicon and the insulator silicon dioxide. These semiconductor structures are called MOS devices. An iridium-metal oxide semiconductor capacitor was described above in dealing with sensors integrating gas-diffusion and detection [63–65], so only a single ISFET design employed in flow systems is discussed here.

Alegret *et al.* devised a pH ISFET based on a flow-through cell designed by themselves and an FI manifold including a gas-diffusion module for the on-line separation of gaseous analytes with acid–base properties. In this way, they obtained a linear determination range of 1×10^{-4} – $1 \times 10^{-2} \text{ mol.l}^{-1}$ for ammonia and 7×10^{-5} – $4 \times 10^{-3} \text{ mol.l}^{-1}$ for sulphur dioxide, with an *rsd* of 1 % and 0.5 %, respectively [99].

6.3 Flow-through chemical sensors based on integrated reaction, separation and detection

With flow-through (bio)chemical sensors based on triply integrated continuous systems, separation processes and reactions take place either sequentially or simultaneously. On the other hand, detection occurs simultaneously with one of the two processes (Figure 16). This type of sensor involves permanent immobilization of the reagent and/or catalyst. Occasionally, however, no active ingredient of the (bio)chemical reaction is immobilized (*i.e.* if the reaction takes place in the solution held in the flow cell). Separation processes can be enacted through membranes (dialysis, gas diffusion) or solid supports packed with beads or coated with a film. The immobilized reagent can play a single or dual role: acting as and ingredient of the derivatization reaction and/or facilitating separation.

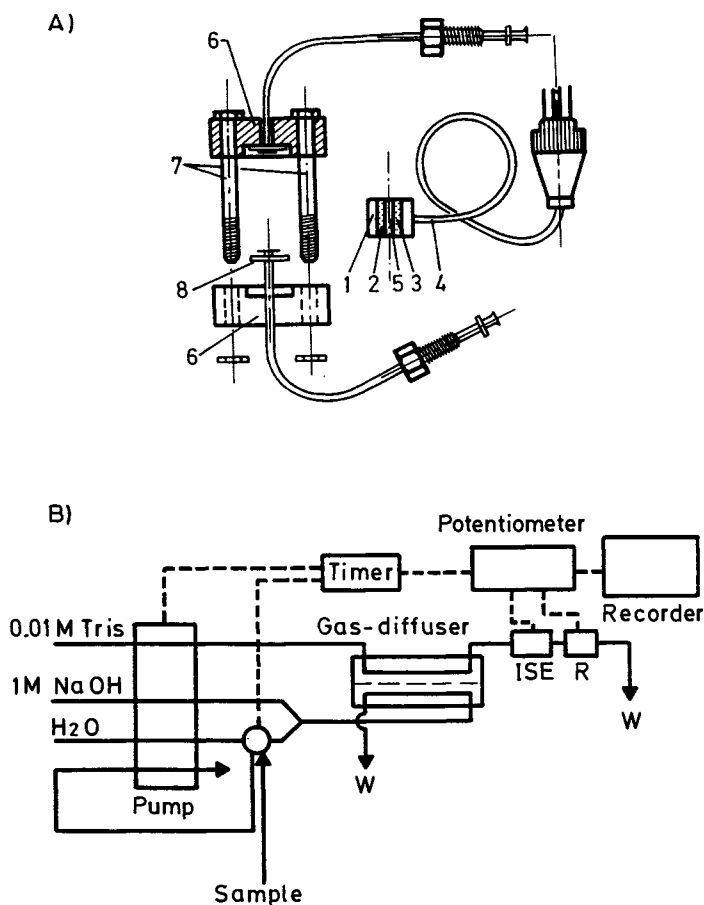


Figure 15: (A) Tubular flow-through electrode: 1, Perspex body; 2, conducting epoxy cylinder; 3, mobile carrier PVC membrane; 4, electric cable; 5, channel (1.2 mm ID); 6, holders; 7, screws; 8, O-rings. (B) Schematic diagram of a system for on-line monitoring of ammonia: ISE, tubular flow-through ammonium ion-selective electrode; R, reference electrode; W, waste. (Reproduced from [98] with permission of the Royal Society of Chemistry).

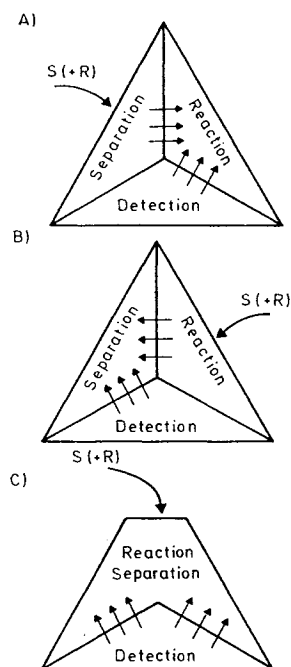


Figure 16: Generic types of (bio)chemical flow-through sensors based on triple integration of separation, reaction and detection. Differences lie in whether the integrated processes take place sequentially (A and B), or simultaneously (C). S, sample; R, additional reagent. (Reproduced from [1] with permission of the Royal Society of Chemistry).

6.3.1 *Flow-through (bio)chemical sensors based on integrated reaction, gas diffusion and detection*

Most sensors based on triple integration conform to the design shown in Fig. 16.A, *i.e.* separation takes place prior to reaction and detection, which are simultaneous.

Freeman and Seitz developed a sensor for oxygen based on the chemiluminescence (CL) of tetrakis(alkylamino)ethylene (EIA). The device consists of a reagent chamber as shown in Figure 17.A. The gas stream passes into the chamber of the reaction housing and the oxygen in the gas can then diffuse across the Teflon membrane to react with the EIA solution to produce the observed CL signal, which is acquired by a photomultiplier tube (PMT) located behind the glass cell. The gases are then vented to the atmosphere. The steady-state CL intensity is proportional to the oxygen partial pressure. The response to pure O_2 decays gradually over a 12-h period. This decay is associated with EIA consumption. The detection limit for O_2 in the gas phase is estimated to be 1 ppm (v/v). The device also responds to oxygen in water, but the slope of the analytical curve is decreased as a result of the slow mass transfer of O_2 through the membrane surface [101].

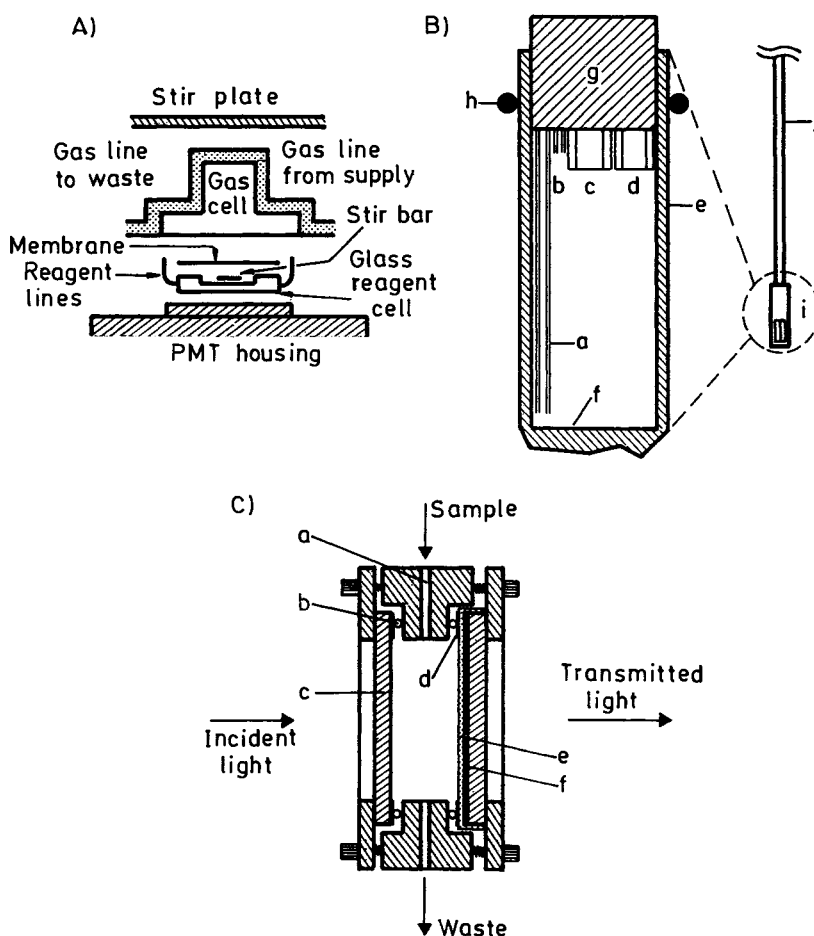


Figure 17: (A) Cross-sectional view of gas and reagent cell for determination of oxygen. (B) Renewable-reagent $p\text{CO}_2$ sensor: (a) reagent delivery capillary; (b) reagent exit capillary; (c) fibre optic from source; (d) fibre optic to detection system; (e) white silicone rubber membrane; (f) white silicone sealant; (g) epoxy; (h) O-ring; (i) sensor housing; (j) fibre optic cable. (Note: individual components are not drawn to scale). (C) Sensor for determination ammonia. (a) sample, (b) O-seal ring, (c) glass plate, (d) gasket, (e) gas-permeable membrane, (f) optode membrane. (Reproduced from [101], [102] and [105] with permission of the American Chemical Society).

A new method based on a renewable-reagent fibre optic sensor, for measuring the partial pressure of CO_2 ($p\text{CO}_2$) in seawater was recently reported [102]. The sensor was constructed from a combination of fibre optics and capillary tubing potted together with a UV-curable epoxy resin (Figure 17.B). The silicone membrane used provided a gas-permeable barrier between the reagent and seawater and also acted as an efficient scatterer for light emitted from the source fibre. The back-scattered incident radiation was collected with fused silica fibre. The reagent flowed through a 1-m 100- μm ID/170- μm fused-silica capillary tube to the fibre tips and exits through a 1-m 75- μm ID/150- μm OD fused-silica capillary. The total sensor pressure drop with these tubing diameters and lengths was *ca.* 2 psi, and the smaller diameter exit capillary helped create more back-pressure to squeeze bubbles out of the gas membrane before they entered the view of the fibres. The sensor operated both in a diffusion-dependent steady state and in an equilibrium regime depending upon the indicator flow-rate. The optimal precision was 0.8 μatm for 300-550 μatm of CO_2 , calculated from the response sensitivity and three times the root mean square noise. Response times (100%) ranged from 11 to 26 min depending on the indicator flow-rate. The sensor performance was tested on a research cruise and the results were compared to the underway $p\text{CO}_2$ measured simultaneously by an infrared CO_2 analyser.

Several ammonium sensors based on gas diffusion membranes placed in a flow cell and fibre-optic reflectance measurements have been reported. Detection is effected by an acid-base indicator that can be used in immobilized [103] or dissolved form [104]. An optical sensor (optode) that responds selectively to ammonia gas in solution was realized by combining a gas-permeable membrane with a plasticized PVC membrane, the latter incorporating a cation-selective neutral ionophore, a H^+ ion-selective neutral chromoionophore that changes its absorption spectrum upon protonation, and a lipophilic site. Figure 17.C depicts the flow-cell used to assess the optode performance. The combined membranes were mounted in the flow-through cell on the detector side of the spectrophotometer. A gasket was inserted between the cell wall and the PTFE membrane to protect the fragile polymer from puncturing. No reference was needed. The optodes based on NH_4^+ ion-selective neutral ionophores of the macrotetrolide type exhibited a high selectivity towards ammonia over its derivatives [105].

6.3.2 *Flow-through sensors based on integrated reaction, liquid-liquid separation and detection*

Sensors based on integration of dialysis (or ultrafiltration), reaction and detection rely on the use of a membrane accommodated in a flow-cell to deliver one of the ingredients of the (bio)chemical reaction directly. Metal ions are determined by using a pressurized membrane through which a spectrofluorimetric reagent is forced into the flow-cell, as in the device proposed by Inman *et al.* [106], where a fluorogenic indicator is forced through an ultrafiltration membrane into the analyte solution. A prototype for application of this approach is shown in Figure 18.A. The membrane is in direct contact with the sample solution, and the sensor body consists of two stainless-steel units connected by an exterior shaft. The upper portion was threaded to receive the same fibre optic wave guide used with the manifold. The

lower portion is used to hold the membrane and ligand reservoir. The distance from the end of the fibre to the membrane can be adjusted from 0 to 2 cm. Pressure to the ligand reservoir is supplied through a length of silicone-rubber tubing attached to a tank holding compressed nitrogen. The most important component of the indicator system is the membrane through which the ligand is forced. The membrane must efficiently restrict ligand transport so the flow can be controlled by varying the pressure on the ligand reservoir. The sensor performance was assessed by measuring the response of the indicator, 8-hydroxyquinoline-5-sulphonic acid, to magnesium ion; the response was linearly related to concentration changes on time scales of 1 s.

Spectrophotometric detection, chemiluminescence development, membrane separation and their combinations were successfully accomplished in a flow-cell such as that shown in Figure 18.B. The ensuing approach combines fibre optics, flow channels, spacers, reflecting surfaces, and separation membranes into a robust sandwich-type unit that comprises two PVC blocks with holes serving as the inlet and outlet for the donor and acceptor stream. An additional hole is made in the block at the acceptor side into which the common end of the bifurcated optical fibre is press-fitted. The spacers placed between the two blocks have slots that determine the width of flow path, while the effective lightpath length is given by the thickness of the spacers. The membrane is placed between two such spacers and the entire sandwich is assembled together by means of two screws. By using more than one spacer at a time, the effective volume of the cell and the lightpath length can be modified. Except for the chemiluminescence applications, a bifurcated optical fibre is used, the light source being a laboratory-made housing accommodating a commercially available halogen lamp. The device was used for pH optosensing, chemiluminescence assays of cobalt(II) and hypochlorite in water, and the spectrophotometric determination of phenol and ammonia [107].

Biosensors based on dialysis/enzymatic reaction and detection have been used in other analytical areas whenever the enzyme concerned could not be immobilized. The most convenient solution in those cases was to deliver the catalyst directly through a membrane accommodated in the flow-cell [108].

No (bio)chemical sensor integrating analytical reaction with liquid-liquid extraction and detection has so far been reported.

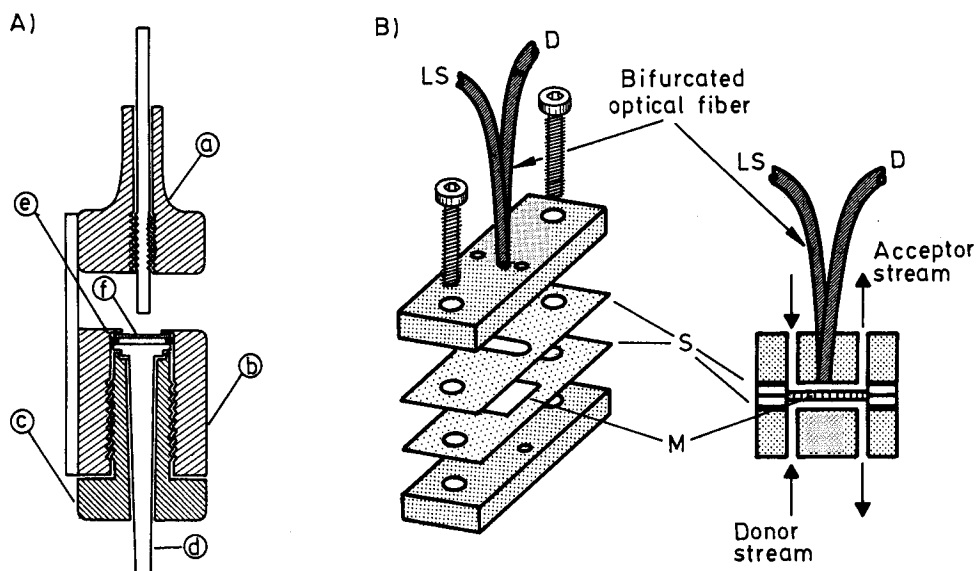


Figure 18: (A) Schematic diagram of a pressurized membrane. (a) Upper portion of probe with fibre-optic wave guide; (b) lower part of probe; (c) threaded reservoir holder; (d) plastic indicator ligand reservoir; (e) rubber o-ring; (f) ultrafiltration membrane. (Reproduced from [106] with permission of Elsevier Science Publishers). (B) Fibre optics flow-through sensor for cobalt, hypochlorite, phenol and ammonia. LS light source; D detector; S spacers; M membrane. (Reproduced from [107] with permission of American Chemical Society).

6.3.3 *Flow-through (bio)chemical sensors based on integrated reaction, sorption and detection*

These are the most numerous on account of the variety of available materials for use as supports (usually for the reagent, which reacts with the analyte on passage through the flow-cell, the product being subsequently retained for measurement).

There are several luminescence sensors based on ionophores permanently immobilized on a support packed in a flow-cell, the functioning of which is illustrated in Figure 16.A. Seitz *et al.* developed one such sensor in which an ionophore (I) selective towards the cation of interest (C) was non-covalently immobilized on particles of controlled pore glass (CPG). A small glass capillary was then filled with the I-CPG phase and placed in the cell compartment of a spectrofluorimeter. As an aqueous mobile phase containing C and 8-

anilino-1-naphthalenesulphonic acid (ANS) was pumped through the capillary, C bound reversibly to immobilized I, the resulting complex then forming an ion-pair with negatively charged ANS on the CPG surface. The ANS fluorescence signal, which was highly quenched in an aqueous solution, increased dramatically for the ANS bound to the hydrophobic CPG surface, and was used to determine the amount of C originally present in the mobile phase. Of all the devices tested, the most promising was the one employing valinomycin on COG for K^+ determination in the presence of a significant excess of sodium and calcium [109].

Ishibashi and co-workers reported a potassium ion optode using Dodecyl-acridine Orange (dodecyl-AO⁺) attached onto a PVC membrane [110]. The positively charged chromophore (AO⁺) of dodecyl-AO⁺ is known to fluoresce more strongly in a non-polar solvent than in a polar solvent. The chromophore moves toward the sample solution by extraction of a potassium ion into the membrane. The polarity change around the chromophore causes a sensor response, *i.e.* the fluorescence intensity decreases as the polarity increases. The potassium ion is selectively extracted into the membrane with a valinomycin ionophore. Therefore, the sensor response is selective towards the potassium ion and reversible as a result of the presence of a long alkyl chain in dodecyl-AO⁺ that retains the chromophore at the boundary between the membrane and the sample solution. However, the fluorescence intensity of dodecyl-AO⁺ gradually decreases with time ($\sim 18\%/h$) owing to its low solubility in water. The sensor was not applied to environmental samples, but only clinical samples such as human blood plasma [111], the matrix of which is as complex as those of some environmental samples, on which the sensor system should perform equally well. The instability of valinomycin can be circumvented by using alternative ionophores including polynactin, a well-known ionophore for ammonium ion that was also assayed by the authors and provided an improved response relative to the previous sensor, which was insensitive to sodium. The sensor is highly responsive to anions as well [112].

Reagents immobilized on solid supports as films or beads that are in turn placed in a flow-cell allow temporary retention (separation) and derivatization to take place simultaneously (Figure 16.C). This approach has been used for the spectrophotometric and spectrofluorimetric determination of metal ions and other chemical species.

The relative humidity of ambient air can have a significant impact on many physical and chemical processes that take place in the atmosphere. It must therefore be monitored in order to obtain reliable predictions for the behaviour of atmospheric pollutants. Several types of sensors have been developed to this end. Most of them use optical fibres coated with a film containing a Co(II) salt. Figure 19.A shows one such device that consists of an optical waveguide coated with a film (anhydrous cobalt chloride suspended and immobilized onto PVC), a light source, a photodetector and an associated electronics package. The waveguide consists of a thin-walled glass capillary tube, one of which ends is rounded off, which aids in focusing transmitted light onto the phototransistors. Clear plastic rods are used to manufacture optical couplers that are bored to accommodate the individual components. A handling system was designed and constructed to control the introduction of vapour and/or diluting air to the sensor, which was regulated by solenoid valves interfaced to a microcomputer [113]. The sensor exhibited very good sensitivity in the relative humidity

range 60-95% and provided an S-shaped curve comparable to that obtained by Russel and Fletcher using a similar sensor [114]. Further developments of these reagent/polymer film may extend the region of reliable response of the device by using polymer/copolymer films of different hygroscopicity or combinations of different metal salts as reagents.

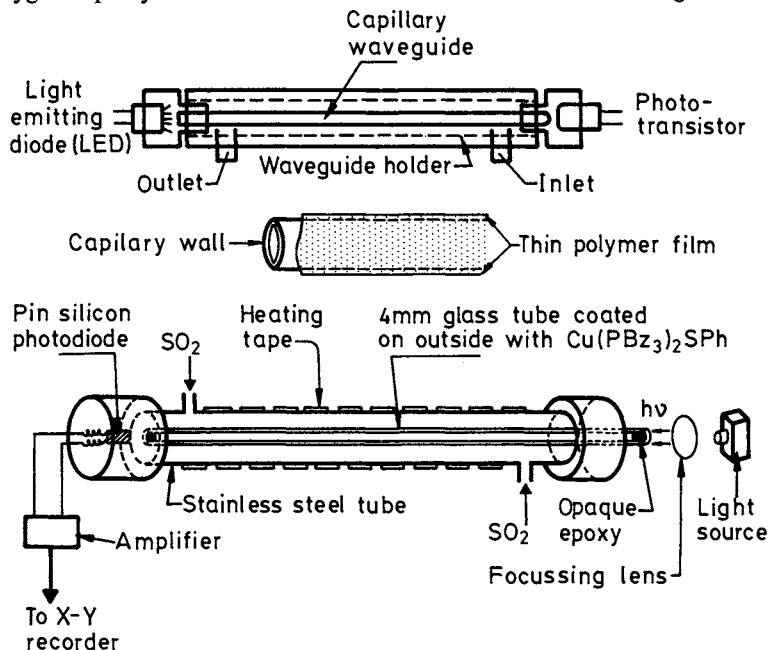


Figure 19: (A) Diagram of an optical sensor for humidity (Reproduced from [113] with permission of the American Chemical Society). (B) Diagram of sulphur optical sensor. (Reproduced from [116] with permission of Elsevier Science Publishers).

A reversible sensor for SO_2 also based on optical waveguides allows this environmental pollutant to be determined at concentrations below 100 ng.ml^{-1} [115]. It consists of a Pyrex tube initially coated with Cu /tribenzylphosphine/thiophenolate complex and holding an unfiltered tungsten-halogen light source at one end (Figure 19.B). Interaction of sulfur dioxide with the initially white complex results in the reversible formation of an orange adduct that is detected by the change in the transmitted light intensity via the photodiode located opposite the light source.

Some reactions with a seemingly low analytical potential can be used to implement sensors of excellent performance such as that based on the Fe(II)/SCN^- system. The configuration shown in Figure 20.A allows the sample to be inserted into a stream of carrier-eluent ($0.2 \text{ mol.l}^{-1} \text{ NaF}/0.5 \text{ mol.l}^{-1} \text{ NaOH}/0.02 \text{ mol.l}^{-1} \text{ NaAcO}/0.002 \text{ mol.l}^{-1} \text{ Na}_2\text{EDTA}$) and subsequently

merged with another of $0.4 \text{ mol.l}^{-1} \text{ NH}_4\text{SCN} + 0.5 \text{ mol.l}^{-1} \text{ HCl}$. By having the latter stream circulate continuously through the flow-cell, which is packed with Dowex 1-X2-200 anionic resin, a constant amount of ligand is maintained on the support. As the sample is passed, Fe(II) forms the well-known red complex with thiocyanate, which is eluted after the whole sample plug has passed through the cell and carrier circulation resumed. This *in situ* concentration achieved simultaneously with reaction and detection gives rise to transient signals that allow analyte concentrations in the range $10\text{--}400 \text{ ng.ml}^{-1}$ to be obtained with a high selectivity. The sensor was applied to various types of samples including waters and wines [116].

An FI manifold similar to that shown in Fig. 10 but using a single ?? rather than channels for the derivatizing reagents was employed for the determination of Cd(II) with a special sensor in which CdI_2^{2-} was retained on WAE Sephadex resin packed in the flow-cell of a photometric detector and a subsequent complex-displacement reaction with 4-(2-pyridylazo)-resorcinol (PAR) monitored via the displacing ligand. Formation of the coloured chelate and detection were simultaneous (Figure 16.A). The method thus developed features a linear determination range between 30 and 500 ng.ml^{-1} of Cd(II) , and an rsd of 1.8 and 3.4% for 200 and 50 ng.ml^{-1} of Cd(II) , respectively. The high selectivity of this sensor was demonstrated in a comprehensive study of interferences [117].

Fibre optic evanescent wave spectroscopy based on AgClBr fibres and a Fourier transform infrared (FTIR) spectrometer was recently used for the first time to measure chlorinated hydrocarbons (CH). A minimum detection limit lower than 10 ng.ml^{-1} was achieved by coating the fibre with low density polyethylene, which was reversibly enriched with CH. The sensor response to CH diffusion through the polymer layer was analysed theoretically and the predictions were found to be in good agreement with the experimental results, which opens up excellent prospects for expanding the available range of detection devices and analytes [118].

One other very promising starting point for sensor design is the use of piezoelectric crystals coated with different organic materials for the sensitive, reversible and reproducible determination of gaseous organophosphorus compounds. The effects of the coating material, concentration and type of compound concerned on sensitivity and reproducibility have been studied and mechanisms for the interaction between the target vapour and coatings proposed [119]. This is thus a potentially vast, still unexplored field for research.

Figure 20.B depicts a multi-sensor for the simultaneous determination of phosphate and silicate based on the formation of their respective heteropoly acids with molybdenum and the use of a manifold in which the flow-cell, packed with C_{18} resin, is accommodated in one of the loops of a 10-port electrically actuated valve. The optosensing system provides for real-time monitoring of the rate of signal change during the reduction step to Molybdenum Blue. A kinetic optosensing method was developed by which the reduction rate difference between the retained heteropoly complexes allows the simultaneous determination of phosphate in the nanogram per millilitre range, and silicate at microgram per millilitre concentrations [120].

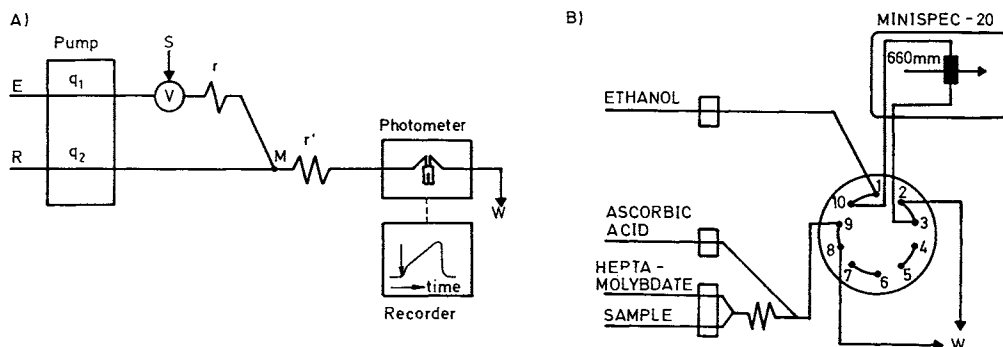


Figure 20: (A) Manifold for implementing a flow-through sensor for iron. S sample; E eluent solution; R reagent; V injection valve; W waste; r and r' reaction coils; q_1 and q_2 flow-rates. (Reproduced from [116] with permission of Elsevier Science Publishers). (B) Manifold for implementation of the optosensing system for the simultaneous determination of phosphate and silicate (Reproduced from [120] with permission of the American Chemical Society).

6.4 Final remarks

The above examples testify to the great potential of (bio)chemical sensors for environmental applications, which they can endow with the sensitivity and selectivity required for each type of matrix through the *in situ* concentration they provide and the kinetic measurements and separations they allow for. In addition, the increased sample throughput, ease of handling, and low purchase and maintenance costs of the experimental set-up required, make these devices worthy of greater attention by environmental R&D officials in order that developments may match achievements in other areas such as clinical analysis; as noted earlier, some of the advances in this field can readily be applied to environmental problems [121].

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7.

Fiber optical sensors applied to field measurements

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In the last few years the expansion of industrial activity has had a considerable impact on environmental air and water pollution. Our increasing knowledge of pollution and its effects has heightened awareness of the importance of good water, soil and air quality and led to the organization of regulatory environmental protection programmes all over the world. These programmes include:

- Treatment of hazardous waste;
- Detection of leakage of fuel or other chemicals into the environment;
- Pollution monitoring of underground, surface and sea waters;
- Evaluation of pollution at short and long distances from major factories and other polluters.

Monitoring requires the continuous quantification of a great number of products or analytes at many different sites [1], and thus the work and cost involved is enormous. Traditional analysis, which implies the problems of representative sampling and subsequent sample stabilization, transport to the laboratory, preparation for analysis, measurement *etc.*, is extremely time consuming and incompatible with the continuous monitoring of certain analytes in environmental analysis. Sampling is the most critical of these steps because of the difficulty of obtaining a representative sample. Furthermore, the stabilization of some analytes, such as sulphide, is not always an easy task and special care must be taken.

Some of these problems could be overcome by performing *in situ* analysis which would obviate the need for a representative sample and avoid the risk of changes in the chemical form and concentration of the analyte during transport and sample pretreatment.

A sensor is a device that is able to indicate continuously and reversibly the concentration of an analyte or a physical parameter [2]. If the signal originates from a chemical or biochemical reaction, the sensor is a chemical or a biochemical sensor. If a physical property is evaluated the sensor is considered a physical sensor.

Sensors have made a substantial and important contribution to *in situ* pollution monitoring. Only those based on the use of optical fibers allow spectroscopic analysis at the site of interest. They allow the analyst to bring the laboratory to the sample instead of the sample to the laboratory as is usually the case, and thus their use may overcome or minimize the above-mentioned problems of analyte instability.

The recent development of microelectronics (digital computing and semiconductor technology) and optical fibers has permitted notable progress in the determination of several analytes of industrial and environmental importance. Several reviews and books on this topic can be found in the literature [2-8]. Chemical sensors based on optical fibers are called optrodes (optical electrode) or optodes (from a Greek word for optical path). The use of fibers as simple light pipes to transmit spectroscopic information to an instrument detector is the easiest way of remote sensing. However, as the number of analytes of interest which are naturally endowed with photometric or luminescent properties is low, an unaided optical fiber is almost impractical as a sensor. A more useful type of fiber optic chemical sensor (FOCS) incorporates a reagent phase, enabling interaction with the analyte of interest. An ideal FOCS should allow *in situ* reversible determination of very low concentrations of contaminants in the environment.

The main advantages of FOCS over other kinds of sensors can be summarized as follows:

- They allow *in situ* determination and real-time analyte monitoring;
- They are easy to miniaturize because optical fibers have very small diameters;
- They are fairly flexible: optical fibers can be bent within certain limits without damage;
- They can be used in hazardous places and locations of difficult access because of the ability of optical fibers to transmit optical signals over long distances (between 10 m and 10000 m);
- Multielement analysis is possible using various fibers and a single central unit;
- They normally permit non-destructive analysis;
- Optical fibers can carry more information than electrical cables;
- Probes are often easy and inexpensive to build.

They also have the following disadvantages:

- The number of reversible reactions is very limited, so in many cases probes have to be regenerated after use;
- Commercial accessories for optical fibers are not standard items;
- The properties of the indicator may vary when it is immobilized;
- They usually have lower dynamic ranges than electrodes;
- In some cases the concentration of the immobilized indicator is unknown and two optodes prepared similarly can have different analytical characteristics;
- The sensor life-time is limited.

Many papers and reviews dealing with the development of FOCS have been published in the literature but only a few have reported applications to environmental monitoring [8]. Therefore, this field is still in an embryonic state and major problems, such as reagent immobilization, the paucity of specific reversible reactions *etc.*, need to be solved.

The key to constructing a successful FOCS is selecting an appropriate chemical reaction that has the specificity, sensitivity and stability required to determine the analyte in the environment. Once the reaction has been chosen, an appropriate support and means of measurement have to be selected.

7.1 Determination of target compounds

Air pollutants may exist in gaseous or particulate form. The former include sulphur compounds, nitrogen oxides, carbon oxides, hydrocarbons *etc.* Particulate air pollutants have diameters varying from less than 0.01 mm to more than 100 mm and may contain both inorganic and organic substances. Some of these pollutants are transported over large distances and oxidized by the atmosphere, hence causing the acid rain problem. Weak acids such as CO₂ and SO₂ strongly influence the pH of rain and other precipitation. Atmospheric concentration of CO₂ of 340 g.m⁻³, at 15°C, produces an equilibrium pH of 5.6 in unpolluted rainwater (boundary pH), while for SO₂ concentrations above 5 mg.m⁻³ in air the pH is below 4.6 [9,10].

The analytes of interest in waters include heavy metals, anions, radioactive waste, organic compounds *etc.* In addition, pH is important to evaluate the acidity of water and thus the possible effect of acid rain.

Acid rain causes the content of dissolved base cations in soil to increase and soil pH to decrease. A significant decrease in soil pH in Central Europe has been detected over the last 20 years [9,10]. Other factors that have also contributed to the decrease in pH are biotic processes and humus accumulation. The deterioration of soils significantly contributes to the worsening of tree health as does a high O₃ concentration. Other important reasons for knowing the pH of reservoirs, flowing water, seawater, *etc.* are because it affects mineral solubility, bioavailability, dissolution kinetics, redox kinetics and because freshwater acidification reduces the fish population. The toxicity of certain analytes (Se, Sb, Cr *etc.*) strongly depends on their chemical form and thus pH also has an indirect effect on the toxicity of some waters. All the factors mentioned above point to the need for FOCS to monitor the pH of waters, including rain water, and in order to detect sharp variations in pH or small continuous increases in acidity [8].

All metal pollutants originating from waste water or industrial residuals are naturally present in the aquatic environment but, when the concentration is higher than usual, presents a threat to biota. Usually concentrations of essential elements are at the level of 1 nmol.l⁻¹ or greater, while very toxic elements are at lower concentration. Pollution can not be evaluated for the presence of strongly toxic elements at high concentration but water is considered polluted when biota are unable to protect themselves against high concentrations. Thus, micronutrient elements such as copper become toxic at elevated concentrations.

The chemistry of some ions is very complex because they are potentially present in different oxidation states in waters. Taking into account that the toxicity may vary with the oxidation state of the element, total content data is not enough. Owing to the interest in continuous monitoring of waters, the development of available sensors to simultaneously determine several ions would represent significant progress and, furthermore, allow the differentiation of possible species. These sensors would overcome the inherent problems related to the lack of species stability, the special care required for sample treatment, *etc.* A special case would be the determination of volatile elements, such as sulphide, for which *in situ* monitoring in waters is necessary.

Remote fiber sensor for monitoring the most important ions would be of great interest but the absence of ions with fluorescent or photometric properties in solution makes the use of remote fiber sensing very difficult. Thus the main principle of these sensors, similarly to pH sensors, is based on quenching effects, photometric or luminescent reactions. There are several sensors for metallic species such as aluminium, beryllium,

sodium *etc.*, but due to the lack of sensitivity and incompletely solved problems they have not yet been applied to analysis in the aquatic environment.

While some gases are a source of environmental contamination, others, such as oxygen, either in air or dissolved in water, are necessary for humans and other living organisms. Oxygen concentration is the main parameter in evaluating water quality and the importance of its determination explains the major effort made to develop FOCS to monitor this dissolved analyte. Nowadays, several FOCS for oxygen allow real time determination of small changes in concentration in the marine ecosystem.

Other important analytes monitored by FOCS are: hydrocarbons, which are released into air mainly through the evaporation of solvents, fuels, the partial combustion of fuels, and leakage from pipes. The chlorinated hydrocarbons, and especially the tri-chlorinated (TCE), are among the most common organic contaminants in groundwater. Other important toxic hydrocarbons are the polycyclic aromatic hydrocarbons (PAHs), which leach into the water supply, *e.g.* from coal-tar pitch used as an internal coating in water pipes.

Pesticides are contaminants of priority interest. Like hydrocarbons, they are difficult to determine because of their great variety. They have a considerable impact on the environment and some are not removed by conventional drinking water treatment. The maximum concentration allowed in water is 0.1 mg.l^{-1} . Pesticides which are properly handled do not normally reach toxic concentrations in the environment, and the main reason for heavy environmental contamination is apparently due to accidents, leaks *etc.* Usually the determination of pesticides in waters requires expensive instrumentation, is impractical outside the laboratory, and is time consuming because of the need for sample preparation and preconcentration. The development of FOCS using specific immobilised enzymes or fluoroimmunosensors is an area of great interest.

The increase in the industrial use of petrol and petroleum derivatives has heightened the risk of leaks from tanks during transport, particularly at sea. Sensors would be an alternative to detect possible leakage and take measures to reduce pollution.

7.2 Validity of sensors

The development of sensors is still at an embryonic stage and major problems still need to be solved. The characterization of a newly constructed sensor involves the testing of several parameters before the detection limit, limit of quantification, linearity of response *etc.* can be calculated.

Response Time. In some sensors this parameter is a function of the analyte concentration. Thus, the response time is not constant over the whole calibration curve. This is quite frequent in pH sensors whose response time at near neutral pH may be higher than at acid or basic pH. The alternative methods proposed for preparing calibration curves are measuring at a fixed time before the equilibrium is reached, measuring at equilibrium or measuring the variation in the slope (change in analytical signal versus time) with concentration. To minimize the delay in response, probes should be designed with low analyte mass transport times.

Sensor Reversibility is the most desirable characteristic because it allows indefinite sensor use and therefore continuous monitoring of the analyte.

Precision is the reproducibility of the method as shown by the agreement of independent measurements under defined conditions. The lack of specific reversible reactions makes it necessary to use disposable probes or big reservoirs (which allow several determinations), or to regenerate the probe after use. All these problems, combined with the inherent irreproducibility of sensor construction, result in considerable irreproducibility of sensor measurements.

Bias is a systematic error inherent in a method or caused by other factors that affect the result. An example of possible bias is the measurement of pH by a reflectance or luminescence sensor in samples of different ionic strength or viscosity. It is important to evaluate all these possible errors before the measurement and to ensure that the solutions for calibration are prepared in matrices similar to those of the samples to be tested.

Cross-Sensitivity is caused by the presence in the sample of substances that may affect the sensitivity of the sensor response to the analyte of interest.

Sensor Lifetime. In many cases immobilized reagents suffer degradation due to the action of light, temperature or solutions used for regeneration. This degradation often occurs very slowly, so the sensor can be used for many measurements, although it has to be recalibrated after a certain number of analyses. To overcome bleaching or other kinds of reagent deterioration, the device focussing the light from the lamp has a shutter to avoid illumination of the sensor for longer periods than are strictly necessary and thus to preserve the active phase of the sensor.

Reproducibility. Two measures of reproducibility can be used. One is the variation in the readings of an individual sensor at different times, the other the reproducibility among different sensors. The latter is estimated by comparing the response of many sensors to the same set of calibrants. Irreproducibility among sensors is a major problem because the immobilization of reagents at different times under the same conditions results in probes with different analytical characteristics. This problem becomes more serious if the solid supports, especially resins, used for immobilization are from different batches.

Once all the above factors have been evaluated, a so-called laboratory test should be performed to ascertain the specificity of the FOCS by testing it with environmental samples or at least with samples matching the composition of environmental matrices.

The evaluation of the FOCS performance should be based on actual environmental samples and should include field simulations. Once proper operation has been confirmed by a sufficient number of independent analyses, the FOCS can be tested in the field.

To check that FOCS have not lost analytical characteristics during storage, they should be recalibrated against at least two sets of calibrants in the field before "in situ" measurements are carried out. The optimum number of tests solutions to be used depends on the precision, bias, linearity required and on the precision between different probes [8].

In order to ensure that the sensor properly fulfils its intended environmental application, it is important to know in advance the required temperature, humidity, airborne particle content, etc. of the real samples to be tested and the cable length.

So far very few sensors have been examined and validated using environmental *in situ* analysis.

7.3 Instrumentation and design

In sensor terminology, "transducer" is the group of materials and chemicals with which the analyte is sensed. The literature describes four basic ways in which the transducer system can modulate the signal transmitted through the optical fiber from the radiation source: intensity, phase, time decay and mode modulation.

In the first type of modulation the intensity of the analytical signal changes with the measured property of the analyte. Many sensors of this type have been reported because there are hundreds of suitable colorants and fluorescent indicators.

These sensors suffer from intrinsic interferences, such as loss of signal through the fiber or the connectors, variation of the intensity of the radiation source and photobleaching of the indicator, which cause errors in the quantification of the analyte. To cope with these interferences the instrumentation used with the sensors must have a reference system.

Sensors based on phase and time decay modulation are not sensitive to intrinsic interferences and do not need reference systems. Most sensors based on this principle are not used to quantify analytes chemically but rather to measure other parameters such as temperature or pressure [11,12].

An ideal environmental monitoring system should be able to quantify low levels of analyte in *in situ* conditions. The instrument used with optical fibers include those designed for laboratory work during sensor development and testing and those designed for *in situ* measurements.

The first optical fiber spectrophotometer used for *in situ* determination of environmental contaminants was designed by Klainer [13] in 1988 and it was transported to the site by truck. To solve the problem of bulk, portable and battery-powered instruments which incorporate technological innovations to reduce their size and weight are being developed.

Instruments designed to work with optical fiber sensors generally have five basic elements:

- a) A low-power pulsed or continuous radiation source in the form of incandescent lamps, light-emitting diodes (LED) or collimated sources, such as lasers;
- b) Narrow-band filters to select the required wavelength;
- c) A low-powered detector, such as solid photodiodes or photomultiplier tubes;
- d) Miniature digital readout devices to display the data;
- e) The optical fiber sensor system itself, known as a probe for irreversible processes.

The configurations of the instruments coupled to optical fibers vary considerably. Figure 1 shows two representative examples, the first with a single fiber and the second with a double fiber. The disadvantages of only one optical fiber include the increased interference due to the intrinsic luminescence of the fiber, the low ability to measure absorbance and the need for an optical splitter.

An important part of the design and construction of optical fiber spectrophotometers is the coupling of the source, fiber and detector to attain maximum intensity of the light entering and leaving the fiber. This can be achieved by a direct contact with the source for example by focusing the precollimated beam from the source into the fiber or using collimated sources such as lasers. If the instrument has a double fiber, it is necessary to use two optical systems to obtain double coupling.

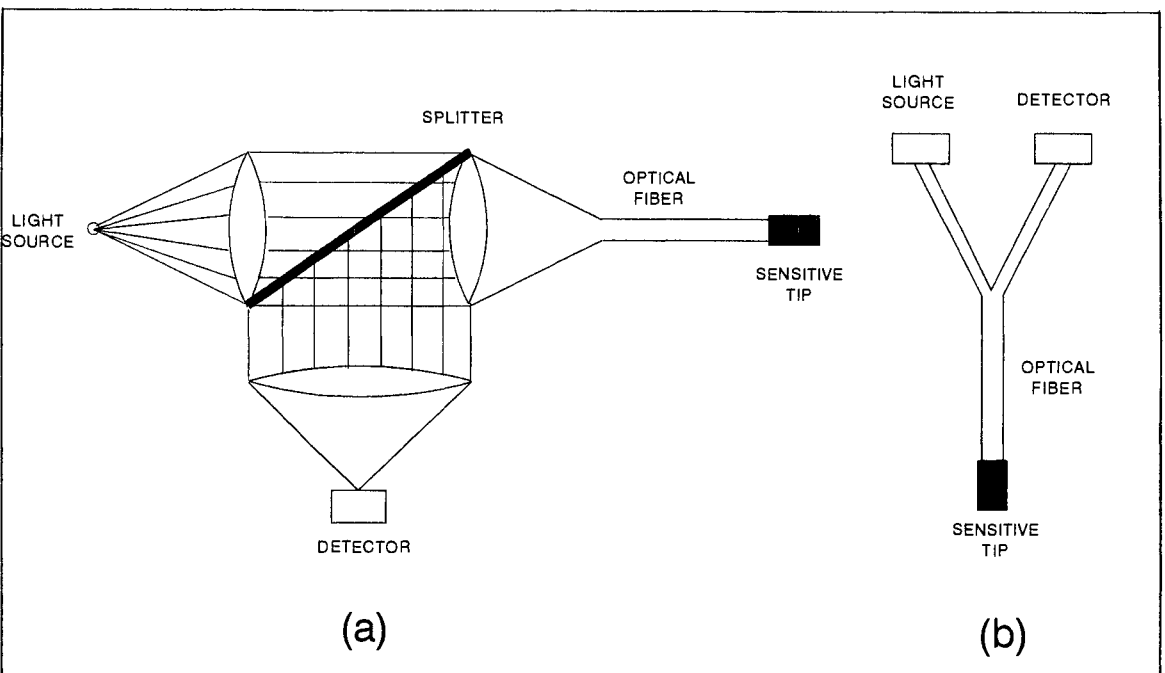


Figure 1: Configurations of FOCS equipment: a) with a single optical fiber and b) two optical fibers.

The above description has been limited to some general considerations; more detailed information can be found in several specialized publications [2,8,14].

The part that distinguishes a classical instrument from a sensor is the FOCS or probe. Once designed, a FOCS looks simple, but the design process is complex because the many steps involved (selection and immobilization of the reagents, selection of the membrane, coupling to the optical fiber *etc.*) require the knowledge of several disciplines (chemistry, physics, optics, spectroscopy *etc.*).

A probe has three main parts: a reactive phase, a membrane and an optical fiber. Sensors can be divided into three groups based on the position and form of the reactive phase, as shown in Figure 2.

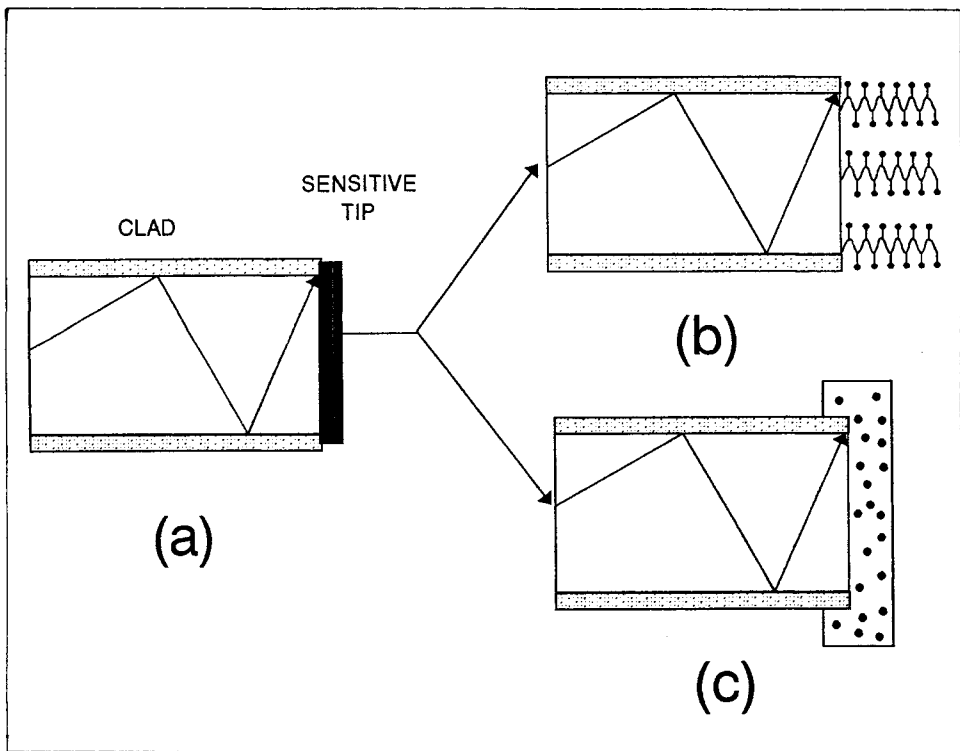


Figure 2: Fiber optic chemical sensor configurations: a) tip-coated FOCS; b) surface-amplified FOCS; c) FOCS with chemistry imbedded in a membrane or porous glass.

In Figure 2, the following configurations are represented:

- a) FOCS with the reactive phase at the tip of the fiber;
- b) Evanescent wave FOCS;
- c) FOCS with the reactive phase on the fiber wall.

Type a) is the most common. The reactive phase is at the tip of the fiber and interacts sensitively and selectively with the analyte of interest.

The main disadvantage of this kind of sensor is the low contact area ($3 \cdot 10^{-3} \text{ cm}^2$), which makes it almost impossible to quantify analytes at very low concentration unless the reaction involved is very sensitive [2,15,16]. Work is underway to improve the sensor performance by making more reagent accessible for the reaction; this can be done by using polymers covered with covalently bound reagent [17] or by confining the reagent in a membrane [18,19] or polymer [20] permeable to the analyte.

Evanescent wave sensors have the reactive phase on one side of the fiber, where the coating has previously been removed. Measurement is in the region of the evanescent wave.

The last option is to put the reagent on the fiber wall, between the core and the coating [21]. The refraction index of this phase must be higher than those of the core and the coating, and the coating must be porous to the analyte. The higher contact area of these sensors facilitates interaction between the reagent and the analyte, so that the analytical signal is superior to that obtained with evanescent wave sensors and terminal reactive phase sensors.

A membrane is used in FOCS to confine the reagent, protect it from the environment or increase sensor selectivity through selective permeation. The membrane can be deposited directly on the fiber from a polymeric solution as a thin layer or can be grafted to the fiber with a covalent bond. The materials normally employed are polymers, such as cellophane, polytetrafluoroethylene (nafion) and polyorganosiloxanes (silicones).

The FOCS used for environmental applications have membranes which are permeable to gases and non-permeable to liquids. For example, Milanovich *et al.* [22] used a thin-layer membrane of polycarbonate and polydimethylsiloxane permeable to CO_2 and non-permeable to protons.

The optical fibers is another essential part of the probe. The material, made of glass, fused silica, plastic *etc.*, must be chosen according to its stability in the environment in which the measurement is made and for its maximum transmission of radiation at the wavelength of interest. The fibers used are normally multimode and have a large numerical aperture to ensure an easy coupling with the source. The diameter most frequently used is between 100 and 600 μm .

The availability of UV-visible transmission optical fibers with low attenuation and background contributions is limited. This problem can be avoided by using very bright light sources (e.g. above 360 nm) and sensitive detectors although this will obviously increase the final price of the monitoring device.

7.4 Water monitoring

Groundwater monitoring is probably one of the main areas of research and application of remote analysis techniques based on optical fibers. Optical fibers offer many advantages in comparison to the classical methods which require the digging of boreholes to collect sample for later analysis in the laboratory. The advantages of optical fibers for groundwater monitoring include [23]:

- The possibility of real-time measurements which facilitate the mapping of contaminant plumes;
- The feasibility of detecting the particular depth at which a contaminant may be present;
- Optical fibers can be arranged along the periphery of aboveground or underground solid waste tanks and connected to an alarm system to detect leakages;
- Hazardous measurements such as those of nuclear wastes or nuclear power plant environments can be performed without danger to the operator.

7.4.1 pH Sensors

Most pH FOCS are reversible and classifiable as: a) absorbance or reflectance and b) fluorimetric sensors. The former use colorimetric acid-base indicators to measure changes in the reflectance or absorbance with pH and the latter use luminescent acid-base indicators. The indicator may be fixed to the end of the optical fiber by adsorption on polymeric supports or covalently bonded to CPG or entrapped into sol-gel glass. Some studies on the immobilization of chromophores by adsorption techniques have been reported [24,25]. Although this technique is very simple, it suffers from the progressive leakage of the chromophore. Immobilization by covalent bonding is more efficient since it guarantees the absence of the reagent leakage. The outlook for CPG bound and sol-gel entrapped indicators [25, 26] now seems to be very promising, despite the influence of CPG and sol-gel porosity on the structural and spectroscopic characteristics of immobilized indicators. These observations concerning chromophores apply not only to pH sensors but also to ion and other types of sensors.

The first pH FOCS was developed by Peterson [27] and is based on the use of phenol red copolymerized with acrylamide and bis-acrylamide microspheres of 5/10 mm diameter. The solid phase is packed in cellulosic dialysis tubing at the end of a pair of optical fibers to monitor changes in absorbance with pH. The sensor was useful in the pH 7.0/7.4 range with a precision of 0.01 pH units. Ionic strength and temperature slightly affect the response of the sensor. The small pH range of the response makes its application to pH monitoring in the environment very limited.

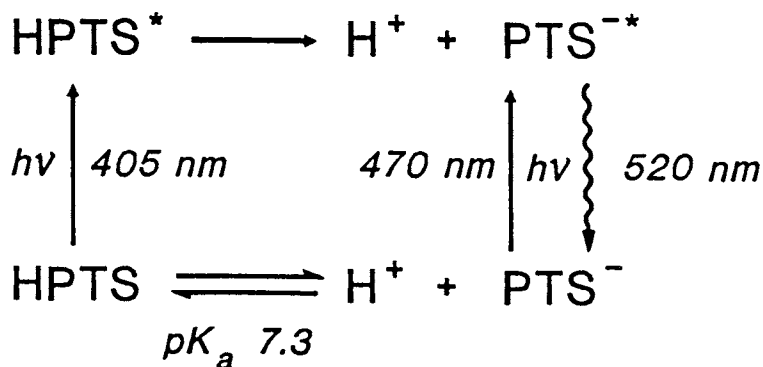
Kirkbright *et al.* [28] described a sensor which measures the reflectance of bromothymol blue adsorbed on styrene-divinylbenzene. The solid phase was placed at the distal end of a bifurcated fiber bundle and retained by a polytetrafluoroethylene (PTFE) membrane. The response of this sensor ranged from pH 7 to pH 9 and was affected by particles in suspension. This was attributed to ambient light-induced reduction at the sensitive tip or absorption of scattered radiation at the membrane solution interphase. Another major source of errors in this sensor is the considerable effect of variations in ionic strength and temperature. The probe response to temperature changes was non-linear from 19 to 50°C. The authors designed a portable instrument using a LED light source and a photodiode as the detector, which is very promising for field measurements.

A similar sensor was developed by Moreno *et al.* [29] using cresol red immobilized on an anionic Dowex IX 10 resin. The sensor has two working pH ranges but only the sensor 6.1-7.2 ranged showed promise for environmental pH monitoring. The device response was not affected by suspended particles or by coloured anions at concentrations below 10^{-3} mol.l⁻¹. Variations in ionic strength up to 10^{-1} mol.l⁻¹ did not significantly affect the response of the sensor. The sensor was calibrated against solutions whose pH

was determined by a conventional pH-meter. The sensor has been used to measure pH in laboratory water but not in the field.

The first reversible luminescence sensor was developed by Saari *et al.* [30]. It is based on fluorescence quenching of fluoresceinamine incorporated into an acrylamide-methylene-bis(acrylamide) copolymer covalently attached to a surface-modified glass fiber. This sensor has a short response time because it does not require a membrane and, consequently, H^+ mass transport is reduced. It has the widest useful response range (pH 2 to 9) because of the two successive pKs of fluoresceinamine. This broad range would make the sensor excellent for field measurement if it were more sensitive and allow for more reproducible measurements.

Another fluorescence sensor, with a response range of pH 6.5-8.5, was developed by Zhujum *et al.* [31,32]. In this probe the trisodium salt of 8-hydroxy-1,3,5-pyrene trisulfonic acid (HPTS) is electrostatically immobilized on an anion-exchange membrane. The excited state of HPTS ionizes more rapidly than it returns to the ground state (Scheme 1). Thus below pH 7.3 the emitted fluorescence intensity is characteristic of PTS^* . Since the excitation wavelengths of HPTS and PTS^* vary from 405 to 470 nm, pH can be determined by measuring the fluorescence intensity ratio of the two excited species. This method eliminates errors from leaching, photobleaching, light source intensity fluctuations and detector drift. Furthermore the pH determination is independent of temperature, ionic strength or other ions. Precision is 0.02 pH units but the membrane life is relatively short and the fairly sophisticated equipment is required.



Scheme 1: Acid based processes of ground-state and photo-excited HPTS [63]

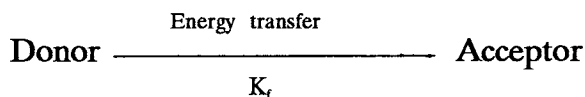
Jordan *et al.* [33] developed a new pH sensor for the 6.0-8.0 range based on energy transfer from eosine, a pH insensitive fluorophore, to phenol red (an absorber). The two reagents were co-immobilized on polyacrylamide. Sensor precision was 0.008 pH units and response time about 10 s. The protonation of phenol red shifts its absorbance maximum to shorter wavelengths and thus reduces absorptivity in the overlap region. The

energy transferred decreases with decreasing pH and the fluorescence intensity rises. Thus pH changes from 6 to 8 are detected by the variation in fluorescence intensity. This sensor is very sensitive, durable and resistant to photobleaching. It has potential for the environment but no data on field trials have been reported.

A system for pH rainwater measurement by optosensing flow injection analysis has been developed by Woods *et al.* [34]. It is based on monitoring the color change at 580 nm of an indicator covalently bound to a commercially available cellulose matrix. An actual probe has not been designed. The method has been validated by using a glass electrode as an alternative technique. A feature of this kind of sensors is that the working pH range can be increased by using several indicators immobilized on a single surface combined with detection at several wavelengths.

A FOCS for sea/water pH monitoring has been reported by Serra *et al.* [35]. It uses phenol red adsorbed on XAD-2, a hydrophobic support which is highly resistance to marine conditions. The response of the sensor has a linear dependence on salinity and temperature in the ranges $12 < S < 42\%$ and $10 < T < 35^\circ\text{C}$, respectively, but beyond these ranges the microprocessing of data is necessary. No method validation or quality control data have been reported for pH measurement in sea/water.

Lakowicz *et al.* [36] employed the advantages of using lifetime or decay time measurements in developing an optical pH and pCO_2 sensor. A two-part sensor has been used:



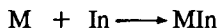
The acceptor displays changes in its absorption spectrum in response to pH or pCO_2 . The mechanism of inducing a pH (pCO_2) dependent change in the donor decay time is the fluorescence resonance. No applications of this sensor have been reported but, considering its analytical characteristics, it appears to have adequate accuracy for clinical use in blood gas determination. Probes such as rhodamine 6G-phenol red, or indocyanine-thymol blue or tris(phenanthroline)rutenium-phenol-red could have wide applications in analytical chemistry.

Recently, Kostov *et al.* have described a method of producing membranes for optical pH sensors [37]. The membranes of neutral red and congo red covalently bonded to a transparent acetylcellulose film have good durability and a short response time. More research on the immobilization of other reagents is needed. A notable feature of this method is that it allows pH indicators and enzymes to be immobilized simultaneously.

Finally, Ge *et al.* [38] have developed a fiber optic pH sensor based on evanescent wave absorption. A pH-sensitive conductive polymer (polyaniline) was immobilized on the core of a silica optical fiber. The pH value can be expressed as a fifth power polynomial function of absorbance at selected wavelength near the IR region to produce very good correlations with values obtained from commercial pH electrodes. Its application to remote sensing is underway.

7.4.2 Sensing cations and anions

The determination of metal cations by optical sensors is usually based on a reaction between the analyte and a ligand and gives rise to a variation in the optical properties of the metal-ligand complex.



The sensor response interval is defined by K_f in the equation $pM = \log K_f \pm 1$. For pM values lower than $\log K_f - 1$ the reagent is saturated with metal ion and there is no further response to increasing concentrations of analyte. In contrast, if pM is higher than $\log K_f + 1$, almost all the reagent is free and there is a negligible response to decreasing analyte concentration.

Certain problems inherent to cation sensors, such as the lack of reagent selectivity and the effect of pH on complex formation, seriously reduce their analytical potential for environmental monitoring. Anion detection is still more complex, with the most promising reactions being those based on ligand interchange. So far, the FOCS for cations have been more notable than those for anions in spite of the lack of reversible reactions.

A reversible UO_2^{2+} fluorescence sensor for ground-water monitoring has been developed [39]. Since UO_2^{2+} fluorescence is higher in acidic medium in the presence of phosphate, a sensor reservoir was incorporated to allow the continuous addition, through a membrane, of 1% phosphoric acid to the sample solution. A laser at 413 nm was used and the lifetime luminescence emitted was observed at 513 nm. This device has been successfully utilized to determine uranyl at concentrations as low as $10 \mu\text{mol.l}^{-1}$ in radioactive groundwater samples.

Nafion is a very promising exchange membrane for sensors owing to its electrostatic and hydrophobic attraction properties that enable the immobilization of reagents such as Rhodamine B as well as those elements that have a quenching effect such as Co(II), Cr(III), Fe(III), Cu(II), Fe(II), Ni(II) and NH_4^+ . Those ions that do not quench the reagent are monitored by measuring the rate speed of increase of fluorescence intensity. A detection limit of 1 mmol.l^{-1} was achieved and the sensor has the attraction that the reagent does not react with the analyte. However, its selectivity is very low [40].

Several sensors based on the use of classical fluorescence reagents immobilized on different supports have been proposed for monitoring cations such as Al, Be, Mg, Zn, *etc.* These irreversible sensors are not selective. Al(III) is the species that has received most attention as an analyte for these sensors.

Saari *et al.* [41,42] immobilized morine on cellulose and placed it at the end of a bifurcated optical fiber to obtain a sensor with a pH-dependent fluorescence response to Al(III) or to Be(II). This irreversible sensor was not selective or sensitive enough to determine these analytes in environmental samples.

Zhujun *et al.* [43] developed a reversible fluorescence sensor for sodium based on ion-pair extraction. The reagent phase is a mixture of the ammonium salt of 8-anilino-naphthalenesulfonic acid (ANS), Cu(II)-polyethyleneimine and a commercial ionophore immobilized on silica. In the absence of sodium the ANS binds to the Cu(II) and fluorescence is quenched. In the presence of sodium, it forms ion pairs with ANS causing an increase in fluorescence. A steady response takes 3 min and is temperature dependent.

Alder *et al.* [44] also developed a reversible sensor for K(I) using crown ethers immobilized on Amberlite XAD-2. The sensor also responds to sodium with a K/Na selectivity ratio of 6:4.

An ammonia fiber optic sensor based on the use of multiple indicators has been proposed by Rhines *et al.* [45]. The sensor is suitable for waste water analysis and has been validated using a conventional electroanalytical technique. In comparison with the electrode, the sensor possesses equivalent sensitivity, similar response times and superior recovery times. A notable feature of the sensor is that particulate material does not have to be removed from the sample because turbidity does not interfere with the analysis. Another noteworthy sensor for the determination of this analyte in groundwater and river water has been developed by Reichert [46]. A pH indicator is immobilized in a separate phase and ammonia diffuses into the membrane and deprotonates the indicator. This deprotonation can be monitored spectrophotometrically. The membrane must be as thin as possible to obtain short response times. The detection limit is 80 nmol.l^{-1} of ammonia and the sensor gives reproducible results for two weeks. Interference by 100 fold-excess bicarbonate and acetate are tolerated. Sensor sensitivity and response time are similar to those of ion-selective electrodes. Another reversible sensor for ammonium was proposed by Narayanaswamy *et al.* [47]. This sensor is based on reflectance measurements of bromothymol blue immobilized on a hydrophobic polymer. It has been used to determine ammonia vapour at $1.5 \cdot 10^{-3} \text{ mol.l}^{-1}$ and dimethylamine was the main interference.

The irreversibility of several cation sensors has led to the development of the flow cell optosensor. Faraldos *et al.* proposed a reflectance sensor for iron [48], Barrero *et al.* a reflectance sensor for Al(III) [49] and another for Fe(III) [50]. The latter has a fluorescence pigment immobilized on controlled-pore glass (CPG); it is almost specific for Fe(III), very sensitive, easy to regenerate and stable, making it an alternative to graphite furnace atomic spectroscopy.

Oliveira *et al.* [51] developed an optosensor based on dithizone immobilized on Amberlite XAD-4 which can determine lead in the $3 \cdot 10^{-7}$ - 10^{-5} range with a limit of detection of $10^{-8} \text{ mol.l}^{-1}$. The sensor was easily regenerated using 0.01 mol.l^{-1} HCl followed by citrate-hydroxylamine solution. This sensor is suitable for both on-line and field applications.

One of the latest cation sensors was developed by Ervin *et al.* [52]. It allows Cu(II) monitoring in water and is based on the use of bathocuprone hydrophobically bound to C_{18} polymeric resin. Slope changes in reflectance with concentration are used for the calibration curve. The sensor is regenerated by a redox process by dipping the sensor in 1 mol.l^{-1} HNO_3 and is rapid, sensitive and selective for a specific metal oxidation state, namely Cu(I). The detection limit is $325 \text{ } \mu\text{g.l}^{-1}$ and its validation needs to be improved. The construction of sensors which respond to specific oxidation states of analytes is an encouraging development in speciation.

The first optical sensor for halides and pseudohalides was described by Wolfbeis *et al.* [53]. It is based on dynamic quenching of the fluorescence emission of glass-immobilized heterocyclic acridinium and quinolinium indicators. Fluorescence decreases with increasing halide concentration and the efficiency of quenching increases in the order: iodide, bromide, chloride. Increasing ionic strength leads to negative deviations of the calibration curve. The detection limits for iodide, bromide and chloride are 0.15 mmol.l^{-1} , 0.40 mmol.l^{-1} and 10 mmol.l^{-1} respectively. Phosphate, perchlorate and nitrate do not interfere up to 1 mol.l^{-1} .

Martinez *et al.* [54] developed a fiber optic sensor to determine sulphide in waste water. It measures reflectance intensity of methylene blue as it is formed by the reaction of sulphide and N,N-dimethyl-p-phenylenediamine hydrochloride in the presence of Fe(III). The dynamic range is $0.05\text{--}0.60\mu\text{g.l}^{-1}$ and the main interferents are thiosulphate, sulphite, alkyl mercaptans and phosphate. The methylene blue immobilized on the Dowex 50-X8 resin is stable for a long time, so measurements can be carried out after the reaction is complete.

A remote sensor for chloride measurement over a distance of 100 m fitted with a sensitive tip containing collodion entrapped silver fluoresceinate and a semipermeable membrane has been developed [23]. In the presence of chloride, AgCl is precipitated and a stoichiometric amount of fluorescein is released and its fluorescence intensity measured.

There are very few chemiluminescent sensors despite their advantages over fluorescence sensors (no excitation light source or spectral separation of exciting and emitted light are required). In 1990 Nakagana [55] described a chemiluminescence sensor in which xanthane dye was immobilized on an ion-exchange resin as the sensitive phase for the continuous monitoring of free chlorine (HOCl) in tap water with no interference from chloramines or other compounds. The method was validated using Fe(II) titrimetry.

Several sensors have been developed for cyanide monitoring. Hardy *et al.* [56] used the first evanescent and irreversible optical probe by coating a fiber rod with poly(vinylalcohol) solution of pycrate which forms a reddish-brown product with cyanide. Latter on Goswami *et al.* [57] developed a fluorescence probe for CN-monitoring in public water waste within the range of $500\mu\text{g.l}^{-1}\text{--}5\text{mg.l}^{-1}$.

7.4.3 Organochlorides, aromatic hydrocarbons and mineral oils

Milanovich *et al.* [58] developed one of the first early-warning FOCS for the long-term monitoring of chloroform in a contaminated well. The sensor was based on the work of Fujiwara and others [59,60,61]. When exposed to several, but not all organochlorides, basic pyridine yields a chromophore to emit at a maximum emission of 597 nm ($\lambda_{\text{exc}}=535\text{nm}$) whose intensity is linearly dependent on the organochloride content of the sample at mg.l^{-1} levels [62]. The main shortcomings of the Fujiwara reaction are loss of pyridine, inability to maintain the pyridine pH, susceptibility to changes in the water content, lack of sensitivity and loss of dye colour, although they can be overcome with a good optrode and fluorimeter design [63].

Two different FOCS based on this reaction have been described for the determination of organochlorides. The first is based on a two-phase chemical system and is depicted in Figure 3 [63]. One end of a polymer-clad optical fiber is partially uncladded and sealed with epoxy resin into a volumetric capillary tube to which a 10mol.l^{-1} KOH (in contact with the stripped fiber) and pyridine are sequentially added. The end of the tube is protected with a membrane (Mylar) that is impermeable to water but permeable to volatile organochlorides.

The possible degradation of the epoxy seal by the action of KOH made it necessary to leave an air gap between the hydroxide layer and the optrode end seal. Long fibers (2.5 mm of exposed length) and large reagent volumes (5 ml) were thus required, which resulted in high irreproducibility during the reagent loading process [63,64]. Nevertheless, the results of some preliminary field tests were in good agreement with those obtained using a gas chromatography method.

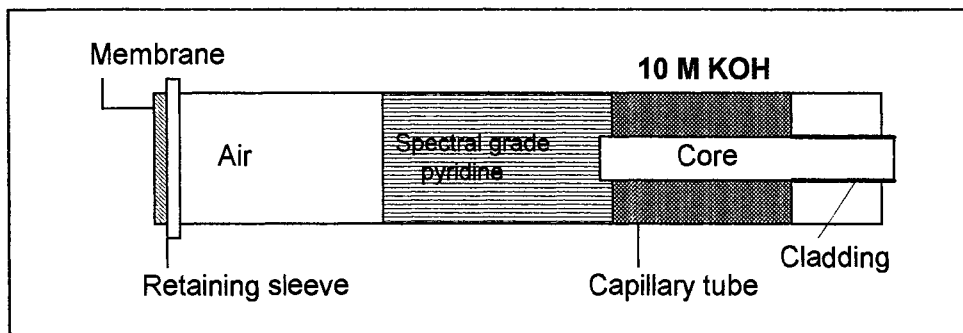


Figure 3: Organochloride fiber optic sensor [63]

These drawbacks are avoided in a sensor featuring single phase chemistry [64]. The tetrapropylammonium hydroxide base in this probe does not react significantly with the epoxy resin, so the air gap can be eliminated and shorter fibers (0.5 mm of exposed length) and smaller reagent volumes (2 ml) can be used. The capillary tube is placed in an epoxy-filled metal ferrule assembly to give a fairly stable optrode. The membrane was also omitted in order to decrease the response time.

For laboratory calibration [8] a sample of chloroform in methanol was spiked into a container half-filled with water ($30 \mu\text{g.l}^{-1}$ – $400 \mu\text{g.l}^{-1}$ in water). The bottle was sealed and continuously stirred for 30 min to attain equilibrium. The chloroform concentration in the gas phase was calculated by Henry's Law (4 to $50 \mu\text{g.l}^{-1}$). Then, the septum seal was replaced by the optrode and the fluorescence was recorded for 20 min. The slope of the fluorescence signal vs time was proportional to the concentration of gaseous chloroform.

A remote fiber optic fluorometer [64, 65] combined with a chloroform optical fiber sensor was used for field experiments at water depths of up to 20 ft. The gas-phase concentration of chloroform in the contaminated well was independently monitored using gas chromatography. The detection limit attained with the optrode was $1.2 \mu\text{g.l}^{-1}$ of chloroform in the gas-phase and the analysis took approximately one fifth of the time required for GC. However, the FOCs response for the aqueous phase was slightly different from that obtained using a GC/MS.

The problems to be overcome before this probe can be widely applied for field measurements are that, under field conditions, the aqueous and gas phase are not in equilibrium, the low viscosity of the reagent causes irregular coupling of the fiber and the fluorescence signal introduces noise into the data. Also the reagent is highly hydrophilic and water quenches the reaction. In recent experiments polymers have been added to the reagent in order to increase its viscosity and decrease its affinity for water [8].

Bürck *et al.* [66] applied near infrared (NIR) evanescent field spectroscopy to quantify different chlorinated hydrocarbon compounds in water at concentrations as low as the $\mu\text{g.l}^{-1}$ level. In general, the application of NIR techniques to the measurement of small amounts of organic compounds in water for environmental purposes is limited by the overlapping of the organic solvent absorption peaks with the broad water OH-absorption band at approximately 1940 and 1440 nm. Nevertheless, this drawback can be overcome by using a quartz optical fiber with a polysiloxane cladding that protects the optical fiber and, because of its organophilic properties acts as a selective layer for the non-polar compounds and prevents interference from water. The sensor consists of an 8-m optical fiber coiled on a teflon support (bend radius 2.5 cm) connected to an optical waveguide spectrophotometer (Guidedwave). It has been applied to determine dichloromethane, trichloromethane and trichloroethylene by immersing the coiled tip in saturated aqueous solutions of these compounds. Scans were run in the spectral region between 900 and 2100 nm where attenuation due to polymer cladding is minimal, and no water interferences were observed.

A linear response to chloroform was obtained in the 78-6765 mg.l^{-1} range for a 400 mm fiber with a detection limit of 18 mg.l^{-1} . Reduction of the fiber radius resulted in increased sensitivity. Response times were about 5.7 min for a 630 mg.l^{-1} chloroform solution and increased with increasing solvent concentration. The sensor is fully reversible and can be used for remote monitoring in contaminated areas.

In a similar approach Krska *et al.* [67] used a silver halide optical fiber coated with low density polyethylene as a fiber optic IR sensor. In this case the fiber (0.9 mm diameter, 100 mm length) was placed in a flow-cell ($V=1 \text{ ml.min}^{-1}$) fitted in the sample chamber of a Nicolet SPC transform infrared spectrometer. Chlorobenzene can be measured at 739 cm^{-1} , and a linear response curve was generated between 5-100 mg.l^{-1} in water after 10 min enrichment. In the case of trichloroethylene the analytical wavelength was 931 cm^{-1} in water. The sensors were fully reversible by drying or rinsing with water.

Kawahara *et al.* [68] described a method for the continuous monitoring of hydrocarbons in water (heptadecylbenzene, dodecylbenzene, n-hexylbenzene, tert-butylbenzene, p-xylene, ethylbenzene, m-xylene, o-xylene, chlorobenzene, 2,6-dimethylstyrene, phenanthrene, 1-methylnaphthalene, 1-phenylnaphthalene, diesel oil and crude oil). The sensor consists of an uncladded fused silica optical fiber chemically treated with different types of silanes (e.g. chlorosilanes and thiethoxysilanes, with octadecyltrichlorosilane resulting in the best performance) to provide an organophilic surface able to adsorb hydrocarbons. This adsorption produces changes in the refractive index at the optical fiber surface which depend on the analyte concentration in the water flow. The optical fiber was placed inside a coiled stainless steel capillary tubing connected to the sample reservoir. A He-Ne laser light source was used, the output signal was focused on a silicon photodiode and the transmission losses occurring over a specified time interval (dB.s^{-1}) were plotted vs the contaminant concentration. The detection threshold was different for each compound and seemed to be related to its solubility in water; for instance, the system could detect 17 mg.l^{-1} of diesel oil in water and crude oil at 3 mg.l^{-1} .

Remote UV-laser induced fluorescence (analyte-instrument distance of 1/25 m) has been applied by Chudyk *et al.* [69] to the analysis of phenol, o-cresol, toluene, p-nitrophenol, 2,4-dinitrophenol and xylenes in groundwater samples, although the following are associated with this system: lower absorptivity and fluorescence quantum yields of the analytes in comparison with those of laser dyes, which are commonly used as tracers [23] to follow pollutant movement; poor transmission of optical fibers in the

UV range where these compounds must be studied; and poor beam quality of pulsed lasers, the only practical sources of UV light. Nevertheless performance was excellent at distances below 25 m. The sensing probe consists of two optical fibers at an angle of 22° held in place by a small grooved aluminium clamp. The device is dipped in a cell containing the sample; fluorescence and scattered light are collected by the optical fiber and guided to the detector. Detection limits (concentration at which the signal is one standard deviation above the background) of 10, 1, and 0.1 $\mu\text{g.l}^{-1}$ have been found for phenol, o-cresol and humic acid, respectively. In the case of unknown contaminants, the technique is potentially selective for different families of compounds but not for individual compounds within a given family.

A portable instrument based on the principles described above has been developed for field testing of surface water and fuel-oil contaminated groundwater [70]. The results were compared with those obtained by the EPA method and by GC/FID, and the authors pointed out the need for more powerful and specific fluorescence methods for molecular detection as well as new and improved methods of field measurement. A second generation prototype instrument built and tested at a later date improved on the performance of the original sensor [71].

In their search for methods of *in situ* detection of trace levels of explosives in groundwater, Seitz and Zhang [72] developed a single optical fiber 2,4,6-trinitrotoluene (TNT) sensor for aqueous samples, based on absorbance measurements. A poly(vinyl)chloride (PVC) membrane containing Jeffamine T403 is held at the tip of the optical fiber by an optical adhesive. Upon exposure to TNT a brown product is irreversibly formed in the membrane in an amount which is dependent on the exposure time, and the ratio of reflected intensity at 824 nm (not affected by colour formation) to that at 500 nm can be related to TNT concentration. A gold grip or a stainless steel strip is used as a reflector to maximize the reflected intensity. The detection limit of this device is lower than 0.1 mg.l^{-1} , although it is limited to sample screening and cannot be used for *in situ* monitoring. The membrane is not sensitive to nitramines, an important class of fairly stable water soluble explosives that may be present in groundwater.

To avoid these disadvantages, a new reversible membrane [73] was prepared using pyrenebutyric acid incorporated into cellulose triacetate plasticized with isodecyl diphenylphosphate. This sensor is based on fluorescence intensity quenching measurements although the authors point out the advantages of using fluorescence lifetime measurements for *in situ* calibration of the optical sensor. Fluorescence lifetimes are not affected by instrumental drift, slow fluorophore losses or time-dependent changes in membrane optical properties which affect fluorescence intensity. The membrane is sensitive to TNT, 2,4-dinitrotoluene (DNT) and hexahydro-1,3,5-trinitro- 1,3,5-triazine (RDX) but the response to all of them depends on the extent of partitioning in the membrane. Also any quencher substance able to penetrate the membrane will interfere and, in fact, oxygen quenching was observed. The detection limits are 2 mg.l^{-1} for TNT and DNT and 10 mg.l^{-1} for RDX, respectively, and the response time (time to establish a steady reading for a given analyte concentration) is about 40 min. The sensor was used for groundwater screening of samples requiring further analysis.

Fluorescence spectroscopy has proven to be a very selective and sensitive analytical method for determining polynuclear aromatic hydrocarbons (PAH) [74] but at the low concentrations present in real samples spectral overlapping may complicate multicomponent PAH analysis. Niessner *et al.* [75] used time-resolved laser-induced fluorescence in a fiber optical sensor developed to determine thirteen PAHs in water

samples. This technique provides additional information to that obtained using fluorescence spectra alone. A pulsed nitrogen laser was used as the light source and the sensor itself consisted of two bare fibers held at an angle of 11° and at a certain distance apart in order to achieve the maximum S/N ratio. Fiber lengths of 50 m were used to check system performance in field determinations. Assuming that the observed emission intensity was the sum of all the contributing fluorescence decay at any given time the authors applied a deconvolution algorithm to the decay curves at different wavelengths for the analysis of multicomponent samples. Detection limits of this technique are in the ng.l^{-1} range.

In recent years much work has been done on the monitoring of oil spills in water. These wastes contain considerable amounts of toxic and carcinogenic hydrocarbons such as benzenes, polynuclear aromatics, amines, phenols *etc.* from different sources such as refineries, shale oil recovery plants, coal conversion operations, chemical plants, ships, offshore drilling platforms, and petroleum-handling facilities. The application of optical fiber sensors for the continuous on-line monitoring of oil contaminants in water could act as an alarm system to announce accidental spills. The intrinsic fluorescence of mineral oils has already allowed them to be determined by airborne laser fluorosensors used to monitor oil and dye spills in the sea [76].

Leaking gasoline from underground storage tanks may contaminate drinking and groundwater and must be detected at an early stage. The monitoring system must be able to measure gasoline reliably and reversibly as a vapour, a liquid or a water emulsion in time to prevent more serious damage. A field instrument capable of monitoring gasoline over a wide dynamic range up to the mg.l^{-1} level has been described [8]. In this sensor a fluorescent dye attached to the tip of the optical fiber, acts as a monochromatic source of light. At the distal end, a refractive index-matched material which is highly selective for gasoline is immobilized on the fiber core. Since air and water have lower refractive indexes than the fiber core and act as a cladding the light returning in the absence of the analyte is very intense and is all propagated through the fiber. When gasoline is present in the samples it couples to the material immobilized on the surface of the core and causes the refractive index to increase. As the refractive index of the cladding becomes similar to that of the core, more light leaks out of the fiber and the returning signal decreases in proportion to the gasoline concentration. The sensor responds to various brands of unleaded standard and super gasoline and only slightly to kerosene and jet fuel. This method could be extended to other compounds provided a highly selective coating was found. A sensor for mg.l^{-1} levels of gasoline is now available from FiberChem Inc, Las Vegas (NV) [7].

7.4.4 Pesticides

Wolfbeis *et al.* [77] have described the working principle of an optical fiber sensor for organophosphorous compounds that is applicable to the remote determination of pesticides in contaminated samples. It is based on the conversion of a red reagent into a blue dye by acetylcholinesterase, whose activity is inhibited by different pesticides. The enzyme is immobilized on nylon beads and placed in a column through which the substrate is passed. The eluate from the column flows to a small cell equipped with one optical fiber to guide incoming yellow LED light and another to transmit outgoing light to the detector. Inhibition experiments with paraoxon showed a response range from $0.2\text{--}200\text{ nmol.l}^{-1}$ at 20°C with a detection limit of 1 nmol.l^{-1} .

Acetylcholinesterase has been immobilized at the tip of an optical fiber using a polyacrylamide gel layer along with fluorescein isothiocyanate-dextran, an indicator that allows the pH changes to be measured during the enzyme activity [78]. At a constant substrate concentration the addition of an enzyme inhibitor reduced the pH variation, an observation which was applied to the determination of the carbamates aldicarb and carbofuran in the 5.10^{-5} - 0.5 mol.l^{-1} and 2.10^{-6} - 0.1 mol.l^{-1} ranges respectively.

A different approach was used by Anis *et al.* [79], who have developed a fiber-optic immunosensor for detecting parathion. Casein-parathion immobilized on quartz fibers selectively adsorbed antiparathion rabbit antibodies although the presence of free parathion interfered with this binding. FITC-goat-antirabbit IgB bound to the antiparathion rabbit antibodies, generating a strong optical signal that decreases in the presence of parathion. The sensor could detect $0.3 \mu\text{g.l}^{-1}$ of parathion.

None of these devices have been applied to environmental samples.

7.4.5 Seawater gases

Almost half of the anthropogenic CO_2 released into the atmosphere is believed to dissolve in the ocean, reducing the planet warming effect of this gas. Therefore much work has been devoted to the development of accurate analytical methods to measure space-time variations in pCO_2 in the ocean and the CO_2 flux at the ocean-atmosphere interface [80] in order to predict the effect of changes in atmospheric CO_2 content on the planet's climate. Walt *et al.* [80] developed a multiple indicator fiber optic CO_2 sensor able to determine low-level CO_2 changes in seawater in the $200\text{-}800 \text{ mg.m}^{-3}$ range with a resolution of approximately $7 \text{ mg.m}^{-3} \text{ CO}_2$. The sensor is based on the inner filter effect (IFE) which allows enhanced pH sensitivity as a result of the screening of the excitation energy (primary IFE) and the simultaneous quenching of emitted fluorescence (secondary IFE) by one or two absorbers with complementary pH profiles and spectral overlap. A mixture of either 7-hydroxycoumarin-4-acetic acid (HCA) and metacresol purple (MCP), or HCA and neutral red (NR) dissolved in synthetic seawater was used as the reactive phase with the first giving the best results. The solution is held at the end of the optical fibers by means of a bisphenol A carbonate copolymer membrane in a specially designed assembly. The main drawback of this configuration is the long response time associated with the diffusion of the gas through the membrane and into the internal filling solution, although it is perfectly compatible with most *in situ* seawater measurements.

Oxygen measurements in aqueous and gas samples have been usually based on the luminescence quenching of a luminophore that can be immobilized or placed behind a gas-permeable membrane at the tip of the optical fibers. The membrane can be made of silicone, poly(vinyl)chloride (PVC), poly(hydroxyethyl)methacrylate *etc.* [81] and will act as a barrier for non-gaseous species, allowing a selectivity increase.

When the decrease of the luminescence occurs because of a dynamic quenching of the luminophore, the Stern-Volmer equation provides a way to calculate the extent of the quenching [81]:

$$I_0/I = 1 + K_{sv}[Q]$$

I_0 and I applied for the luminescence intensity in the absence and in the presence of the quencher respectively; K_{sv} is the Stern-Volmer constant and $[Q]$ is the quencher concentration.

The use of FOQS for oxygen monitoring offers several advantages over the application of the amperometric electrodes such as the Clark electrode: small size, lack of oxygen consumption and their independence from stirring flow rates or the high external pressures that occur, for instance, in seawater studies.

A wide variety of luminescence indicators have been applied in oxygen FOQS, for example, polycyclic aromatic hydrocarbons, longwave absorbing dyes or metal complexes. Several reviews have been published in this area [3,81-83].

Dissolved oxygen is an important parameter to determine the water quality as it is necessary to support all life in aquatic environments. Most of the FOQS for oxygen described in the literature could be applied for this type of measurements. McFarlane and Hamilton [84] were probably the first to apply the principle of oxygen quenching of the fluorescence intensity of an excited luminophore to the determination of oxygen in seawater. The indicator was immobilized at the tip of the optical fiber and when seawater was monitored, the optical components, computer batteries, and other equipment were mounted on a rack in a pressure housing. The sensor gave results that correlated well with those of a YSI oxygen probe, a Clark type polarographic cell commonly used for laboratory and field measurements. The response time was much shorter than that of the YSI probe. Other sensors have been applied for seawater monitoring [85,86] showing comparable results to those obtained with the Winkler titration method.

7.5 Air pollution

The pace of development of optical fiber device for detecting toxic gases in the environment is continuously increasing for the reasons given previously in the introduction to this chapter. The specialized literature on this topic [4, 7, 8, 87] includes many works on FOCS that are potentially applicable to the determination of air borne contaminants although few have been applied to real samples. Thus, sensors have been developed for SO₂ [88], H₂S [89], CO [90] and CO₂ [91] *etc.* that await application in practical environmental studies.

Most of the methods described to continuously monitor gases in air are based on the measurement of an optical property intrinsic to the gas, normally its absorption in the wavelength range between 200 nm (UV) and 4 μ m (IR), as for *e.g.* CO, methane, NO₂ *etc.*, in which case, the analytes can be sensed with a plain fiber.

Laser spectroscopy has been applied traditionally to the remote detection of environmental contaminants. Laser and optical fiber technology offers great advantages over traditional methods, as is evident in the case of low-power laser sources coupled to optical fibers.

If the analyte has no intrinsic optical property (O₂, CO₂, SO₂) it is necessary to have an immobilized reagent in the fiber to interact with the gaseous species. Sometimes the interactions are irreversible so the sensitive terminal is designed to be disposable; in the literature such sensors are referred to as probes.

7.5.1 Carbon monoxide and dioxide

Carbon monoxide absorbs strongly in the NIR and IR regions. The absorption in the IR region (4.66 μ m) cannot be exploited with fiber devices due to the high cost of the fibers able to transmit in that region, the low transmission efficiency and the overlap with the water absorption band.

There are FOCS described in the literature based on the use of reagents sensitive to CO immobilized on porous glass discs; the reflectance signal emitted ($1.4\text{--}1.7\text{ }\mu\text{m}$) is directed through an optical fiber to the detector [90,92].

The carbon dioxide content in the atmosphere is less than 0.03 %, but it increases rapidly owing to human activities. The continuous measurement of CO₂ is an important requirement for the control of emission sources or the maintenance of controlled atmospheres, such as those of tunnels, parkings *etc.* Carbon dioxide has traditionally been quantified electrochemically with CO₂ selective electrodes or by IR absorption spectrophotometry. It cannot be monitored directly with optical fibers owing to their low transmission in this region of the spectrum.

Most CO₂ sensors are based on the same principle as the electrochemical measure, namely that of the pH variation in a buffered solution due to the presence of CO₂. Detection using optical fiber is by means of a pH-sensitive "optical transducer", the indicator can be colorimetric [93] or fluorimetric [22,91,94-97]. These sensors have the advantages of reversibility, but in order to prevent evaporation of the buffer solution or the colorant, all of them are externally coated with a hydrophobic gas diffusion membrane, which leads to a slow response time.

Kawabata *et al.* [98] designed a thin optical film sensor for CO₂ without a membrane and without an inner buffer. The sodium salt of the fluorescent indicator fluorescein is dispersed in polyethyleneglycol and deposited on the optical fiber creating a very thin polymeric layer ($10\text{ }\mu\text{m}$). The response range is up to 28 % (v/v) for CO₂, with a detection limit of 0.1 %. The response is rapid but affected by changes in relative humidity.

Recently, Mc Murray *et al.* [99,100] replaced the soluble polymeric membrane with plastic water-insoluble polymers. Their sensor has been applied to artificial atmospheres of CO₂ content from 0 to 5 % (v/v). The response and regeneration times are less than 3 s.

Wolfbeis *et al.* [96] developed a fluorescence sensor for O₂ and CO₂ based on the use of two sensitive layers. One of them contains tris(bipyridine)ruthenium(II) as an oxygen sensitive reagent and the other HPTS for CO₂ monitoring. Both indicators show the same excitation wavelength.

All the optical fiber devices described in the up-to-date literature have been applied to the determination of CO₂ in water [94], synthetic mixtures of gases [91,96,97] or blood [101], but none have been used to monitor this analyte in air environmental samples.

7.5.2 Nitrogen oxides

NO and NO₂ are the main contaminants of the atmosphere, where they are in thermodynamic equilibrium in the presence of oxygen. In automobile exhausts NO₂ is present in a lower proportion than NO and can be monitored easily due to its absorption band in the visible region at 405 nm, with a detection limit of 20 g.m^{-3} . The device uses an argon laser radiation source [102] and has been applied successfully to monitor NO₂ in car exhaust gas [103]. Unfortunately, NO has no absorption maximum in the visible region but only in the IR one ($5.32\text{ }\mu\text{m}$), where it is well known for overlapping with the water absorption band. Also the transmission power of optical fiber in this region of the spectrum is low. The NIR band is probably the better choice for remote spectrometry using plain fibers, but the sensitivity is very poor.

Nitrogen oxides are dynamic deactivators of the fluorescence of some polycyclic aromatic hydrocarbons, but determination based on this property are unreliable owing to interferences from other gases present in the samples, such as O_2 and SO_2 . Also the estimated detection limit are not good. Low concentration ($1-10\text{ g.m}^{-3}$) of toxic gases such as Cl_2 , HCl and NO_2 [104] can be detected by virtue of the quenching of tetraphenylporphyrin incorporated into Langmuir-Blodgett films. The fluorescence response is reversible for NO_2 , but the indicator must be regenerated for other gases by exposing the film to ammonia. Assays were only performed on synthetic gas samples.

7.5.3 Hydrogen sulphide and sulphur dioxide

Narayanaswamy *et al.* [89] have described a flow-through optosensing method for hydrogen sulphide analysis based on changes in reflectance (580 nm) when H_2S gas reacts with lead acetate impregnated in paper. The detection limit is 50 mg.m^{-3} but the response is affected by flow rate and humidity. The method enables sensitive real-times analysis of hydrogen sulphide and can be used for remote sensing. This sensor is irreversible. It has not been applied to real samples, the same as all other methods using fibers so far published for the optical sensing of H_2S [54] in the environment.

Sulphur dioxide is one of the most common and harmful air pollutants. It can be determined continuously in air by fluorimetry owing to its strong intrinsic fluorescence (330 nm), but this approach has not been adapted to fibers.

Wolfbeis and Sharma [88] described an optical fiber sensor based on the dynamic quenching by SO_2 of benzo(b)fluoranthene immobilized in a silicone polymer and placed on the tip of a bifurcated optical fiber. Interference by oxygen is considerable, although negligible for SO_2 levels below 6% in air at constant oxygen pressure because the quenching efficiency of SO_2 is about 26 times higher than that of oxygen. The detection limit for SO_2 in air is 84 g.m^{-3} (0.01% v/v) and no validation of the sensor has been reported.

The same authors improved the sensitivity of the sensor (detection limit of 10 g.m^{-3}) using a two-fluorophore system, in which pyrene acts as donor and pyrene as acceptor, dissolved in a silicone matrix [105]. Deactivation by SO_2 is more efficient when the energy is transferred from pyrene to pyrene than when only one fluorophore is used.

7.5.4 Ammonia

Ammonia gas can be determined directly with optical fiber by measuring its absorption in the NIR ($1.18-1.67\text{ }\mu\text{m}$) [106] or in the IR region ($10.6\text{ }\mu\text{m}$) [107]. The methods are not sensitive and the detection limit is around 35 g.m^{-3} in air using cells of 19.5 cm pathlength [107].

David *et al.* [108] designed an irreversible FOCS for NH_3 in air based on the well-known reaction between this analyte and ninhydrin. The optical fiber was covered with a solution of ninhydrin poly(vinylpyrrolidone) and the changes in the absorption of the evanescent wave were measured. The probe is able to detect down to the level of 50 mg.m^{-3} .

A large group of ammonia optical sensors is based on the pH changes that this analyte produces due to its basicity. These changes can be monitored with pH indicators (colorimetric or fluorometric) placed in the fiber. These sensors are reversible, but they have not been applied to the monitoring of gaseous NH_3 in atmospheric environmental samples. Narayanaswamy [47] described a sensor of this type to determine NH_3 vapour based on the pH changes of the indicator dye bromothymol blue immobilized on a

hydrophyl polymer support and attached to the end of a optical fiber in contact with an atmosphere containing ammonium vapour in the $1.5 \cdot 10^{-3}$ - $6.0 \cdot 10^{-3}$ mmol.l⁻¹ range. This reversible sensor is based on reflectance measures. The major interference was observed in the presence of dimethylamine and the response was not affected by variations in the concentration of atmospheric water vapour.

7.5.5 Methane

The presence of methane gas in the air is very dangerous because it is highly explosive in proportions higher than 5%. Thus methods are required to detect down to 500 g.m⁻³ of this analyte under normal pressure and temperature conditions.

Methane is routinely determined in mines and in the surroundings of city gas containers. The methods of monitoring methane based on direct measurement with optical fiber of its absorption band in the IR ($3.39 \mu\text{m}$) are not used nowadays due to interferences by water and other hydrocarbons such as ethane or propane and to the high cost and high attenuation of optical fibers in this region of the spectrum. However, the detection limit of 60 g.m⁻³ [109] is suitable for methane monitoring.

The measurement of absorption in the 1.3 - $1.5 \mu\text{m}$ range is much more feasible [110] due to the much higher transmission of radiation through the fiber in this wavelength range. These systems have been applied to sense 0.25% methane in air over several kilometers with a response time of 1.5 s [111], to detect remote leakage of city gas [112] and to monitor methane in a coal mine [113]. This latter device showed a close correlation with conventional methane meters. The detection limit depends on pressure and on fiber length.

Another sensor reported in the literature to determine methane is based on the formation of alkane hydrates which are adsorbed onto the glass and modify its refractive index and thus the fiber transmission properties. The system works with a LED type radiation source that emits in the visible region (560-660 nm) [114].

Finally, another sensor to detect methane is based on the highly exothermic reaction between this hydrocarbon and platinum or palladium in the presence of oxygen. When this reaction occurs at the surface of an optical fiber covered with platinum or palladium, the heat of the reaction produces a phase lag in the light beam transmitted by the fiber, enabling detection by interferometry [115].

7.5.6 Other air pollutants

The advantages of optical fiber sensing are widely known, but they are especially relevant to the detection of organic solvent vapours, because the fibers do not have electric contacts and there is no risk of explosion in a vapour-rich atmosphere.

In 1988, Posch *et al.* [116] developed a sensor capable of optically monitoring the vapours of polar solvents such as ethers, alcohols, esters and ketones. It is based on the reversible decoloration by these vapours of the blue thermal printer used in plotters. The sensitive terminal is placed on the tip of an optical fiber and the variation in the diffuse reflectance is measured. This approach has been tested only with vapour mixtures prepared by bubbling air through the solvent. The detection limits vary from 10 to 1000 g.m⁻³ depending on the kind of vapour tested.

Halothane vapour has been detected by measuring its deactivator effect on the fluorescence emitted by several PAHs [117]. CO, CO₂, N₂O and fluorane do not interfere. Oxygen interference can be avoided by using a second sensor sensitive only to oxygen, making it possible to determine down to 4 % of halothane. The authors mention the

potential application of this sensor to the monitoring of anaesthetic gases in breathing circuits but did not apply it to real samples.

Hydrogen cyanide has been detected in air by oxidation with chloramine T impregnated on XAD-7 resin beads placed in front of the fiber bundle [118,119]. The cyanogen chloride product reacted with 4-picoline(4-methylpyridine) and barbituric acid, giving a colour change detected by measuring the absorbance at 530-565 nm. In air, 1 g.m⁻³ of HCN was detected within 1 min. The device is not reversible, so it cannot be used to monitor HCN in air.

7.6 Conclusions

The application of FOCS for environmental monitoring is still restricted to some analytes such as organic compounds in groundwater, gases or pH.

Some problems associated to the lack of reversibility, selectivity, reproducibility and signal drifts must be solved before the FOCS can be widely applied for field analysis. Thus only a few sensors have been validated to date for this purpose.

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8.

Chromium speciation in environmental and biological samples

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Chromium is ubiquitous in nature, occurring under various chemically, physically and morphologically different forms.

Most surface waters contain very low levels of chromium (1 to a few $\mu\text{g.l}^{-1}$), except for waste waters coming from industries. Industries dealing with paints, pigments, dyes, mordant, rubber, plastics, ceramics, textiles, leather, steel and its alloys, as well as welding activities, smelter works and chrome plating plants are an important source of discharge of the metal, either as Cr (III) or Cr (VI), into the environment causing chromium pollution. The leaching of sanitary landfills also leads to substantial amounts of chromium in ground water.

Measurements of chromium in aerosols from remote areas show its background levels to range from 0.01 ng.m^{-3} to 1.3 ng.m^{-3} [1]. There are however numerous sources of chromium emission including kilns, smelting furnaces, boilers, evaporation from leaching and plating tanks as well as cement production. The burning of oil, coal and wood and the incineration of municipal refuse and sewage sludge also contribute to atmospheric chromium. Chromium and its compounds occurring in the work environment are associated with processes such as welding and grinding of stainless steel, chrome plating, tanning, wood preservation, painting and pigment production.

A detailed overview of the sources and uses of chromium is given by Nriagu and Nieboer [1].

8.1 Different species of Cr and their toxicity

Chromium belongs to the transition group elements from group VI B and can therefore occur in each of the oxidation states from -2 to +6 which are of unequal stability. Only the 0 (elemental), +2, +3 and +6 states are common. The divalent Cr form is unstable in most compounds as it is easily oxidized to the trivalent form by air or by H^+ .

In natural and waste waters chromium exists essentially in its trivalent and hexavalent form. Cr(VI) may be present in aqueous solution as monochromate, bichromate or hydrogen chromate, depending on the pH and the chromium concentration in solution. Cr(III) has the tendency to form a large number of kinetically inert complexes, mainly the hexa-coordinated $[\text{Cr}(\text{H}_2\text{O})_6]^{3+}$ ion. At higher pH, $\text{Cr}(\text{H}_2\text{O})_5\text{OH}^{2+}$ and $\text{Cr}(\text{H}_2\text{O})_4(\text{OH})_2^+$ are also present. At pH 4 and above hydrolysis of the coordinated H_2O occurs, which leads to the formation of OH bridges. Solutions containing Cl^- , SO_4^{2-} and SCN^- can form simple complexed species with Cr(III). This is also the case with several organic ligands such as humic acid. The chromium distribution in natural waters is also controlled by redox processes; oxidation of Cr(III) to Cr(VI) can occur in the presence of solid MnO_2 , while environments rich in Fe(II) and organic matter favour the reduction of Cr(VI) to Cr(III). The oxidizing ability of Cr(VI) in aqueous solutions depends on the pH; at moderate to high pH, the Cr(VI) ions are stable against autoreduction, but at low pH (below 4) Cr(VI) can autoreduce to Cr(III). The different forms of Cr(III) and of Cr(VI) as a function of pH are given schematically by Stern [2].

In indoor air at the workplace, welding fumes are a class of samples which contain unpredictable forms of hexavalent chromium in a complex matrix. The composition of welding fumes depends on the welding technique [3].

In biological systems Cr(III) is the prevalent chromium species. Cr(VI) is easily reduced on entering the body. The occurrence and importance of chromium in the functioning of biological systems were thoroughly studied by Guthrie [4]. The mechanisms of chromium metabolism, genotoxicity and carcinogenicity are extensively described by De Flora and Wetterhahn [5].

The health effects of Cr(III) and Cr(VI) are so fundamentally different that they must be considered separately. A valid generalisation of the biological effects of chromium as an element can therefore not be made. Trivalent chromium is essential for man in glucose, lipid and protein metabolism [6]. The hexavalent form is considered to be toxic because of its ability to oxidize other species and its adverse effects on lung, liver, kidney. Inhalation of hexavalent chromium may cause bronchial carcinomas. Compounds of hexavalent chromium readily penetrate biological membranes and are easily reduced under physiological conditions to trivalent chromium through interaction with essential constituents of the cells, including genetic material. Skin ulceration and irritative dermatitis are also related to contact with chromium compounds. They are responsible for the high incidence of occupational contact dermatitis among, for instance, bricklayers working with wet cement. Wet cement is abrasive owing to its alkaline property and contains Cr(VI) compounds. Chromate is the precursory allergen as it is able to penetrate skin in contrast to Cr(III), while the Cr(III) formed might be the actual allergen [7].

The maximum allowable concentration for chromium in drinking water is $50 \mu\text{g.l}^{-1}$ (European Community Directives 80/778/EEC, L229/20, D48). In occupational health the OEL (occupational exposure limits) for water soluble and certain water insoluble compounds in indoor air is limited to 0.5 mg.m^{-3} for chromium, to 0.5 mg.m^{-3} for Cr(III) and to 0.05 mg.m^{-3} for Cr(VI) which reflects the different toxicity of both species. Hexavalent chromium is such a potent carcinogenic agent for the respiratory tract that continuous monitoring is imposed (Directive 90/3941/EEC on exposure to carcinogenic substances).

8.2 Cr speciation in water

Because of the different toxicity and bioavailability of Cr(III) and Cr(VI) a determination of the total chromium content does not give full information about possible health hazards. Hence, monitoring of the concentration of the separate chromium species is necessary. This also enables us to understand the behaviour of chromium in the environment, e.g. aquatic systems, and to follow the pathways for interconversion.

The first and major obstacle encountered in the development of a reliable method for chromium speciation is preserving the original speciation state of the sample. Sampling, separation and preconcentration procedures may disturb to a significant degree the equilibrium between various species in solution. One of the greatest difficulties results from the redox equilibrium between Cr(III) and Cr(VI). Its great sensitivity to changes in pH and the addition of oxidizing or reducing agents must be considered in every step of the sample treatment until the species separation has been carried out. Sample pretreatment using strong acids and heating shortens the lifetime of Cr(VI), particularly when organic compounds or other reductants are present in the sample and should therefore be avoided.

Two different approaches for the speciation of chromium can be distinguished: simultaneous determination of both species, or determination of one of the species "through difference". The latter means that the concentration is calculated from the total chromium content and the determination of one of the species. This may involve some risk due to cumulative errors. Simultaneous determination of both chromium species decreases such risks but may entail more development work.

8.2.1 Methods of final determination

Determination of the low concentrations of chromium in natural water samples requires analytical techniques with a sufficiently high sensitivity and selectivity. This is even more the case when trying to determine the ultra trace levels of the individual species. Many different analytical techniques have been applied: flame and electrothermal atomic absorption spectrometry (FAAS and ETAAS), spectrophotometry, chemiluminescence, inductively coupled plasma atomic emission spectrometry (ICP-AES), direct current plasma atomic emission spectrometry (DCP-AES), inductively coupled plasma mass spectrometry (ICP-MS), isotope dilution mass spectrometry (IDMS), neutron activation analysis (NAA) and electrochemical techniques. Only a few are species-specific. Therefore they have to be combined with a separation of the species under investigation from the original matrix. The separation techniques include chelating and ion exchange resins, solvent extraction with different organic media, coprecipitation and selective volatilisation.

Some typical literature data on the analysis and techniques employed in Cr(III) and Cr(VI) determinations are summarized by De Jong and Brinkman [8], Katz [9] and by Sperling *et al.* [10]. A summary of the methods most commonly used nowadays, completed with useful modifications and some advantages and pitfalls will be given in the following paragraphs. The detection limits mentioned are calculated as 3 x standard deviation on the blank. This overview is an attempt to describe the present state of the art concerning chromium speciation in different materials and should not be considered as complete.

8.2.1.1 Atomic Absorption Spectrometry, Atomic Emission Spectrometry

Atomic absorption spectrometry using either a flame or a graphite tube as the means for atomization and atomic emission spectrometry with various excitation sources (inductively coupled plasma and direct current plasma) are the most frequently used techniques for the determination of chromium. The detection limits of FAAS, ETAAS and ICP-AES for simple aqueous chromium solutions are respectively $3 \mu\text{g.l}^{-1}$, $0.08 \mu\text{g.l}^{-1}$ and $3 \mu\text{g.l}^{-1}$. The techniques are element specific but lack specificity with respect to the oxidation states of chromium. Those spectrometric techniques coupled with appropriate separation procedures allow the selective determination of tri- and hexavalent chromium. Most of the separation methods described below are combined with AAS or AES as detection methods.

8.2.1.2 Chemiluminescence

The chemiluminescence (CL) reaction of luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) is one of the best known, very sensitive and therefore most applied CL reactions. The detection limit for aqueous chromium solutions is around $0.05 \mu\text{g.l}^{-1}$. Several metal ions have a pronounced catalytic effect on the oxidation of luminol by hydrogen peroxide in basic aqueous solutions. The intensity of the light emitted is proportional to the metal concentration and can therefore be used for quantitation of the element. Free Cr(III) is known to be very sensitive in the catalysis of the luminol oxidation by peroxide, in contrast to Cr(VI), which does not produce any chemiluminescence. Interferences by several other ions that catalyse the luminol reaction are possible. By adding EDTA, most of the interferences can be avoided. The kinetics of Cr(III) to form a complex with EDTA are much slower than for other ions which allows specific complexation of the interfering ions. Applications of the luminol reaction are given by Seitz *et al.* [11], Hoyt *et al.* [12], by Bowling *et al.* [13] and by Williams *et al.* [14].

Chemiluminescence methods in combination with flow injection were recently developed and provide the opportunity for rapid and selective determination of Cr(III). Methods were described by Gammelgaard and coworkers [15] and Escobar *et al.* [16].

Huizenga and Patterson used chemiluminescence to distinguish between chelate bound (oxalate, phthalate, salicylate and tartrate) and unbound Cr(III) and to monitor the rate of reaction of Cr(III) with those carboxylate ligands and with humic acid over a wide concentration range [17,18]. They recommend the method of standard additions for the analysis of unbound Cr(III) in natural samples containing high levels of organic matter to correct for quenching and light absorption.

Besides luminol chemiluminescence, flavin mononucleotide in cationic micellar media was used by Oshima *et al.* [19], and lophine chemiluminescence was described by MacDonald *et al.* [20].

8.2.1.3 Electrochemical methods

Of the existing electrochemical techniques, voltammetry is the most popular for the determination of chromium. Total Cr has been determined directly using polarography or stripping voltammetry but those techniques sometimes lack sensitivity for the low concentrations encountered in natural waters [21]. Higher sensitivity can be obtained by cathodic stripping voltammetry (CSV) preceded by adsorptive collection of a complex of Cr(III) with e.g. diethylenetriamine pentaacetic acid [22] on a hanging Hg drop electrode.

Boussemart *et al.* [23] optimized the cathodic stripping voltammetry method for determination of the different chromium species (including Cr(VI) and total Cr) in seawater. The measurements were preceded by adsorptive collection of chromium complexed with diethylenetriamine pentaacetic acid on a hanging mercury drop. The different behaviour of Cr(VI) and Cr(III) towards CSV allows the determination of reactive Cr(III) and Cr(VI). Total dissolved chromium, including organically bound chromium, was determined after UV irradiation of the sample at neutral pH.

Elleouet *et al.* [24] proposed a method for the determination of Cr(VI) based on chelation of Cr(VI) with diphenylcarbazide (DPC) followed by adsorption of the complex on a hanging mercury electrode. The reduction current of the accumulated chelate was then measured with differential pulse voltammetry. They obtained a detection limit of $0.02 \mu\text{g.l}^{-1}$ in aqueous media.

Amperometry allows determination of the different Cr species without previous separation. The technique is however not element specific which means that other elements or species *e.g.* Fe(III) that are reduced at the potential used for the reduction of Cr(VI) will interfere. Cr(VI) was determined by Pratt and Koch [25] by flow injection amperometry at gold and at iodized platinum electrodes using H_3PO_4 as a supporting electrolyte to suppress the interference of Fe(III). Dissolved Cr(III) and O_2 did not interfere. The detection limit was $5 \mu\text{g.l}^{-1}$.

Electro(lytic) deposition can also be used to preconcentrate chromium prior to detection with another analytical technique, *e.g.* ETAAS, thus replacing the stripping step used in stripping voltammetry. Vidal *et al.* [26] used mercury-coated pyrolytic L'vor platforms as macro electrode for the selective preconcentration of Cr(III)/Cr(VI). Speciation of Cr(III) and Cr(VI) can be done on the basis of the electrolysis potential (E_c): at pH 4.7 and $E_c = -0.30 \text{ V}$, only Cr(VI) is reduced to Cr(III) and accumulated as Cr(OH)_3 by adsorption on a mercury film, at $E_c = -1.8 \text{ V}$ both Cr species are accumulated.

8.2.1.4 Isotope Dilution Mass Spectrometry (IDMS)

IDMS is an analytical technique that combines high accuracy and precision with good sensitivity (detection limits for aqueous solutions are $0.01 \mu\text{g.l}^{-1}$ for Cr(III) to $0.02 \mu\text{g.l}^{-1}$ for Cr(VI)). A great advantage is the use of an internal isotope enriched standard for calibration and that total reproducible yields in the chemical separation and isolation procedures are not required [27]. IDMS is applicable to elements that have at least two stable (or quasi-stable) isotopes which are available with an artificially altered isotopic composition. Chromium has four stable isotopes (masses 50, 52, 53 and 54); ^{52}Cr and ^{53}Cr are used for IDMS analyses.

Nusko and Heumann [27] determined Cr(III) and Cr(VI) by positive thermal ionisation IDMS after separation of the two chromium species. The method is further described in the section of separation techniques.

8.2.1.5 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ICP-MS is not species specific but it offers the advantage of being a very sensitive technique (detection limit for simple aqueous chromium solutions $0.02 \mu\text{g.l}^{-1}$) which lends itself very well to coupling with chromatographic separation methods. In general, the coupling of liquid chromatography requires careful selection of the chromatographic

conditions because organic solvents and high salt concentrations in buffers can significantly affect the ICP-MS performance [28].

Of the four stable chromium isotopes, only those with masses 52 and 53 can be used for ICP-MS; measurements using ^{50}Cr and ^{54}Cr generally suffer from interferences from high background counts due to $^{36}\text{Ar}^{14}\text{N}^+$ and $^{38}\text{Ar}^{16}\text{O}^+$ respectively. The ^{52}Cr isotope is the most abundant (83.8%) and is therefore preferred. Interferences can occur with samples with a high carbon content (of both organic and inorganic origin) or sulphur content because of excessively high background counts from $^{40}\text{Ar}^{12}\text{C}^+$ and $^{36}\text{S}^{16}\text{O}^+$.

Roerhl and Alforque [29] compared ICP-MS with the more classic DPC colorimetry as a detection method for Cr(VI) after separation with ion chromatography. Ion chromatography effectively separated chromate from potentially interfering anionic species (carbonate, sulphate and chloride) that occur at high concentrations in environmental samples. Ashley [30] investigated the polyatomic interference of $^{40}\text{Ar}^{12}\text{C}^+$ on the ICP-MS determination of chromium using the ^{52}Cr isotope.

8.2.1.6 Neutron activation analysis

Total chromium can be determined by irradiation with thermal neutrons yielding the long lived ^{51}Cr radionuclide ($T_{1/2} = 27.7\text{ d}$; E_γ 320 keV). A sufficiently high sensitivity can be obtained by choosing an appropriate irradiation time and neutron fluency (e.g. an irradiation of 14 days at a neutron fluency of $10^{14}\text{ cm}^{-2}\text{ s}^{-1}$ yields a sensitivity as low as 0.01 to 0.005 ng chromium). Neutron activation analysis however is not species specific, moreover, the valency of Cr can be changed during the irradiation due to a Szillard-Chalmers process, which necessitates a pre-irradiation separation of the different chromium species. Chromium determinations using neutron activation analysis can suffer from the nuclear interference of iron. In samples containing a lot of iron, the $^{54}\text{Fe}(n,\alpha)^{51}\text{Cr}$ reaction induced by fast neutrons, also yields ^{51}Cr radio activity leading to an overestimation of the chromium content in the sample. This interference can however be corrected for. As it is not advisable to use liquid samples under the irradiation conditions mentioned, those separation techniques that yield solid samples e.g. co-precipitation are preferred.

Radio tracers can be a great help in the development of a separation method because they allow very simple measurements of the radio activity without the need for tedious sample preparation and avoiding contamination hazards.

8.2.1.7 Spectrophotometry

A variety of spectrophotometric methods with different sensitivity (detection limits mostly of a few $\mu\text{g.l}^{-1}$) exist for the determination of chromium in various environmental samples. An overview of the most popular ones is given by Chackraborty and Mishra [31] and by Sperling *et al.* [10]. They involve the formation of a complex with the chromium species that absorbs in the visible or UV region. In the past selective reagents have been used to distinguish between different oxidation states of certain elements. Most of them however suffer from interferences from either other coloured substances, from substances that also form complexes with the chosen reagent or from oxidizing-reducing agents. It is therefore recommended to combine the determination with one of the separation methods given later in this chapter. In addition, to assure good reproducibility, the working conditions must be kept strictly constant.

The standard spectrophotometric method for the selective determination of Cr(VI) is based on the formation of a complex with diphenylcarbazide (DPC) which has an absorption maximum at 540 nm [32]. A major disadvantage of the DPC method is that interferences by other coloured species such as Fe(III) or Cu(II), or species forming complexes with DPC such as V, Mo and Hg are possible.

Jørgensen and Regitano [33] investigated the possibility of adapting the reaction with DPC and flow injection analysis (FIA) for routine water analysis. De Andrade *et al.* [34] developed a procedure for on line oxidation of Cr(III) to Cr(VI) followed by detection of the produced Cr(VI) as the DPC complex.

Ruz *et al.* [35] combined the findings of the former authors for simultaneous and sequential determination of Cr(VI) and total chromium in water.

Shaopu and Fuchang [36] developed a highly sensitive spectrophotometric method for the determination of Cr(VI), based on the oxidation of I^- to I_3^- by Cr(VI) in acid medium, followed by the formation of a 1:1 ion association complex of I_3^- with a basic xanthene dye in the presence of polyvinyl alcohol.

8.2.2 Separation methods

8.2.2.1 Solvent extraction

The different solvent extraction procedures were reviewed by Rao and Sastri in 1980 [37]. Most of them are more or less specific for one of the chromium species. Only a few deal with Cr(III) extraction due to the inertness of its complexes and the very low ligand exchange rates.

A frequently used method for preconcentration and separation of chromium is the extraction based on the complexation of chromium with ammonium pyrrolidine dithiocarbamate-methyl isobutyl ketone (APCD-MIBK) or diethyl dithiocarbamate-methyl isobutyl ketone (DDTC-MIBK). Actually, the APCD-MIBK extraction, followed by determination of hexavalent dissolved chromium by FAAS is one of the methods proposed by the US Environmental Protection Agency (EPA) [38]. Subramanian [39] explored the feasibility of direct complexation of Cr(VI) as well as Cr(III) without the need to convert the latter to Cr(VI). In general the chelate extraction of Cr(III) under normal conditions is difficult. The authors investigated several parameters affecting the extraction efficiency of both species. Optimized conditions of the concentrations of phthalate buffer and APCD, pH and extraction time allow the selective determination of Cr(VI) in the presence of Cr(III). Interferences from foreign ions were tested and found to be negligible. The humic acid concentration in the solution to be extracted for Cr(III) should be $\leq 2 \text{ mg.l}^{-1}$.

De Jong and Brinkman [8] developed a method for selective and quantitative extraction of Cr(VI) with Aliquat 336 (a mixture of methyl trialkyl ammonium chlorides with alkyl groups, mainly C8-C10) from weakly acidic (pH 2) sample solutions. Cr(III) is successfully extracted from neutral solutions (pH 6-8) containing at least 1 mol.l^{-1} thiocyanate. Cr(VI) does not interfere in the Cr(III) determination and vice versa. Alternatively, total chromium is determined by oxidation of Cr(III) to Cr(VI) with ammonium persulfate followed by Aliquat 336 extraction. The method was applied for the determination of Cr(III)-Cr(VI) in seawater. The thiocyanate method gave a lower Cr(III) result than the oxidation method, probably because the powerful oxidation

method released Cr(III) from colloids and highly stable complexes with organic ligands which were unaffected by the thiocyanate method.

Chakraborty and Mishra [31] describe a very selective extraction method for Cr(VI) with N-hydroxy-N,N'-diphenylbenzamidine from HCl solution into CHCl_3 , followed by colour sensitization with DPC. This method has been extended for determination of total chromium. For this purpose, the sample is oxidized with KMnO_4 before extraction. The Cr(III) content can be calculated through difference.

Beceiro-Gonzales *et al.* [40] report a procedure for the separation of Cr(III) by complexation with 8-hydroxyquinoline and extraction into MIBK. The optimum pH of the aqueous phase before extraction was found to be 8. The complexation reaction, which is very slow, was accelerated by working at elevated temperature in a microwave-oven. The organic phase is measured by ETAAS. Through the difference with the total chromium content, the Cr(VI) could be calculated.

A somewhat different approach of solvent extraction was presented by Mugo and Orians [41]. The volatile trifluoroacetylacetone (HTFA) derivative of Cr(III) is formed via solvent extraction with toluene and detected by electron capture gas chromatography. Total chromium is determined after reduction of Cr(VI) to Cr(III) with sodium sulphite. Because the complexation kinetics of Cr(III) with HTFA are very slow at room temperature (3 to 4 h are required for quantitative extraction), the temperature was increased using a microwave oven.

A similar method, combining the formation of the volatile trifluoroacetylacetone-chromium complex with ETAAS, was used by Arpadjan and Krivan [42].

8.2.2.2 Chromatography

This paragraph deals with the preconcentration/separation techniques based on ion exchangers, chelating resins and immobilized functional groups on solid sorbent materials used on-line as well as off-line. Chromatography in combination with element specific detectors is probably one of the most promising approaches in speciation analyses especially because of its ease of combination with flow injection analysis. The chromatographic separation of Cr(III) and Cr(VI) can be based on the cationic behaviour of Cr(III) and the anionic behaviour of Cr(VI). It is however dangerous to accept this without restriction since Cr(OH)^+ , the predominant form of Cr(III) in *e.g.* sea water, and Cr(III) sorbed on negatively charged colloids in river water are also retained on anion exchange resins.

Pankow and Janauer [43] were amongst the first to report the application of this principle for the determination of both species in natural water. Cr(VI) is adsorbed on a AG1-X4 (strong basic anion exchanger) column while Cr(III) passes through the column without adsorption. Cr(VI) is then eluted with $1 \text{ mol.l}^{-1} \text{ NaCl}$, or with an Fe(II) solution in the so-called reactive elution mode, and measured by FAAS. The authors mentioned difficulties such as losses of Cr(VI) due to reduction on the column and deterioration of the column material through oxidation by Cr(VI).

The former method was refined by Johnson [44] who used the AG1-X4 resin for the selective preconcentration of Cr(VI) and disposable extraction columns filled with aromatic sulphonic acid silane for the collection of Cr(III). The efficiency as well as the selectivity of the columns used is reported to be pH dependent; Cr(III) for instance also binds on the anion exchange material above pH 4.5, probably due to negatively charged

Cr(III) hydrolysis species. The pH should therefore be kept under control. Johnson concluded that the ion exchange methods can only be used to preconcentrate free and labile Cr(VI) and Cr(III) species; colloidal or organically bound fractions can not be determined easily.

Urasa and Nam [45] investigated the separation of Cr(III) and Cr(VI) in natural and waste water using a strongly acidic cation exchange resin with a lithium citrate - oxalic acid solution as mobile phase as well as using a strong basic anion exchange column with HNO_3 as mobile phase. Detection was done by DCP-AES interfaced with the column.

Arar and Pfaff [46] worked on the development of a new EPA method for the determination of Cr(VI) in drinking water, ground water and industrial waste water effluents. The method consists in separating Cr(VI) from the bulk of the material by ion chromatography (Dionex IonPac AS 7 separation column) with an ammonium sulphate-ammonium hydroxide solution as eluent. DPC was used as post column reagent. The coloured complex was detected at 530 nm.

Activated alumina offers the possibility to preconcentrate/separate Cr(III) and Cr(VI) based on the fact that it can function both as a cation or anion exchanger depending on the pH of the solution. The acidic form of alumina has an affinity for anionic Cr(VI) while the basic form of alumina has an affinity for cationic Cr(III). This was used by Cox and McLeod [47] for the development of a simple field sampling technique for the collection of natural water samples. The microcolumns, filled either with acidic or basic alumina, were dipped into river water and a fixed volume of water was drawn through. They were then inserted into an FI-ICP-AES system for elution and quantitation. Elution of Cr(VI) was done with $2 \text{ mol.l}^{-1} \text{ NH}_4\text{OH}$ while Cr(III) was eluted with $2 \text{ mol.l}^{-1} \text{ HNO}_3$. The method has the advantage that the identity of the species can be preserved better as it avoids problems associated with stability and transportation of liquid samples.

Acidic activated alumina was used by Sperling *et al.* [10] for the on-line selective sorption of Cr(III) and Cr(VI). They were preconcentrated sequentially under carefully selected pH conditions. A Clark-Lubs buffer solution at pH 2 for sorption of Cr(VI) and at pH 7 for Cr(III) was used. Interferences by competing anions and cations were found to be negligible at levels normally encountered in natural waters. The flow injection system was coupled to FAAS.

Recently, Sperling *et al.* [48] developed a flow injection method where Cr(VI) is selectively preconcentrated on a C18 bonded silica reversed phase column using NaDDTC as chelating agent. The Cr(VI) chelate is eluted with ethanol, transferred directly onto the platform of a graphite furnace and detected by ETAAS. Total Cr was determined after oxidation of Cr(III) to Cr(VI) by potassium peroxydisulfate and the Cr(III) estimated through difference.

An innovation by Williams *et al.* [14] simultaneously uses two separation columns (connected in parallel) coupled with the highly sensitive chemiluminescence reaction as a detection system. The separations were carried out on a HPLC CG2 cation exchange column for Cr(III) and a AG 4A anion exchange column for Cr(VI). They were eluted with 0.085 mol.l^{-1} and 0.003 mol.l^{-1} potassium sulphate respectively at pH 3. The elution conditions were chosen to give retention times for Cr(III) and Cr(VI) that were different enough to result in two resolved peaks. In order to yield a chemiluminescence signal, Cr(VI) had to be reduced. This was achieved post column by an aqueous stream of potassium sulphite.

A similar procedure was used by Gammelgaard *et al.* [15] who developed a method for the simultaneous determination of Cr(III) and Cr(VI) in a flow system. Cr(VI) passes through the cation exchange column while Cr(III) is delayed for about 2 min. When the sample has passed the column, it is carried into a stream of potassium sulphite which reduces the Cr(VI) to Cr(III). Just before reaching the detector the stream is mixed with a hydrogen peroxide/luminol mixture which results in the chemiluminescence of the sample.

Syty *et al.* [49] described a selective method for the determination of Cr(III) and Cr(VI) based on ion-pairing HPLC coupled to FAAS. They used tetrabutylammonium phosphate as the ion pairing reagent selective for Cr(VI).

The method was adapted by Posta *et al.* [50] for automated on-line separation and determination of Cr(III)/Cr(VI) by high performance flow flame atomic absorption spectrometry (HPF-AS). They chose tetrabutylammonium acetate as the ion pairing reagent. In HPF-AS the aerosol generation of the sample is achieved by means of hydraulic high-pressure nebulisation.

Andrle and Broekaert [51] optimized a HPLC method for the simultaneous determination of Cr(III) and Cr(VI) in waste waters. Cr(III) and Cr(VI) were complexed with APDC, extracted into ethyl acetate and separated by HPLC. The different fractions were detected with UV spectrometry.

Mazzucotelli *et al.* [52] used the liquid ion exchanger Amberlite LA-1 for the Cr(III)-Cr(VI) separation and ETAAS as method of detection. The liquid anion exchanger solution was prepared by stirring 50 ml HCl (6 mol.l⁻¹) with 100 ml of Amberlite LA-1 and diluting it to 250 ml with MIBK. Under these conditions Cr(VI) was completely extracted into the organic phase whereas Cr(III) remained in the aqueous phase. Interference effects due to the presence of foreign ions were investigated by adding increasing amounts of salts of Al, Ca, Fe and Mg to distilled water samples and were found to be negligible.

Amberlite LA-2 was also used by Dyg *et al.* [53] to differentiate between Cr(III) and Cr(VI) in the development of a candidate reference material for chromium speciation in water samples. ⁵¹Cr labelled Cr(III) and Cr(VI) were used for the optimization of the method. The work is further described in section 8.5 of this chapter.

The method was adapted for IDMS by Nusko and Heumann [27]. An exactly known amount of spike solution, a HCO₃⁻/CO₂ buffer of pH 6.4, containing both Cr(III) and Cr(VI) enriched in ⁵³Cr, was equilibrated with the sample. After separation Cr(VI) was re-extracted from the organic phase into an aqueous phase. The most efficient system was an NH₃ solution. After electrolytic deposition of chromium, the isotope ratio ⁵²Cr/⁵³Cr was determined by thermal ionisation mass spectrometry.

8.2.2.3 Coprecipitation

Coprecipitation is an established technique for preconcentration/speciation of chromium. The use of many reagents has been described in the literature. Hydroxides of Fe(III) and Al(III) will coprecipitate Cr(III) efficiently, and sulphates of Pb or Ba can be used for collection of Cr(VI). Similarly carbamates have been applied for coprecipitation of Cr(VI) but gave rather low yields according to Lavi and Alfassi [54].

The coprecipitation of Cr(III) with Fe(III) hydroxides is most commonly used. The method however suffers from the partial adsorption of Cr(VI) onto the precipitate. Another drawback is that the high amount of Fe or Al present must be removed prior to determination with spectrometric methods to avoid interference problems. In addition, Nakayama and co-workers [55] reported that various organic compounds present in natural waters, including humic acid, lower the efficiency of Fe(III) hydroxide as a carrier for Cr(III) presumably by complexation of the latter. This was confirmed by Eckert *et al.* [56] who correspondingly studied the kinetics of reduction of Cr(VI) in natural waters by fulvic acid. They used ^{51}Cr labelled Cr(VI) which was separated from the reaction mixture at chosen intervals. The separation consisted of co-precipitation of Cr(VI) with cobalt tetramethylene dithiocarbamate followed by γ -measurement of the carrier precipitate.

Chromium speciation in waters by coprecipitation with Pb sulphates followed by ETAAS was outlined by Obiols *et al.* [57]. The method allows determination of four Cr fractions *e.g.* Cr(VI), Cr(III) (free), Cr(III) (complexed) and the particulate element. The sample was filtered ($0.45\ \mu\text{m}$) and the precipitate treated with dilute HNO_3 to dissolve and determine particulate chromium. The filtrate was used to determine both Cr(III) and Cr(VI) after coprecipitation with lead phosphate at pH 6-7 and Cr(VI) after coprecipitation with lead sulphate at pH 3.

Lan *et al.* [58] developed a two step coprecipitation method for the determination of Cr(III) and Cr(VI) based on their different coprecipitation behaviour with Pb(PDC)_2 (PDC = pyrrolidine dithiocarbamate) as a function of pH. First Cr(VI) was coprecipitated at pH 4 followed by coprecipitation of Cr(III) at pH 9. Total chromium was determined after reduction of Cr(VI) with NaHSO_3 , followed by coprecipitation at pH 9. The separation was followed by neutron activation analysis for quantitation.

8.3 Cr speciation in welding fumes

The importance of chromium speciation to identify carcinogenic Cr(VI) in air has been mentioned above. A particular case is the monitoring of welding fumes. An acceptable method must provide for collection on suitable materials followed by complete leaching of all Cr(VI) from the sample matrix without liberating any latent oxidizing or reducing agents and must permit a quantitative determination of the unaltered original Cr(VI) concentration.

Sampling, storage and analytical procedure entail the possibility of changing valences of chromium. Reducing or oxidizing substances, such as SO_2 , NO_x , O_2 , O_3 , Fe^{2+} (magnetite) and interfering substances, must be taken into account since they tend to cause erroneous results.

The reduction of Cr(VI) on the filters used for collection of welding fumes is possible. Cellulose nitrate, cellulose acetate, PVC, PTFE and glass fibre filters have been widely used. All the materials reported have been found to reduce Cr(VI) under different circumstances. Binderfree glass fibre filters showed reduction of Cr(VI) to a lesser extent than the others [59].

The method for Cr(VI) determination in welding fumes recommended by the US National Institute for Occupational Safety and Health (NIOSH) uses s-diphenylcarbazide after leaching the filters with either sulphuric acid or 3 % Na_2CO_3 and 2 % NaOH [60]. According to Thomsen and Stern [3], the method has some drawbacks for complex samples such as welding fumes. The low pH enhances the reduction of Cr(VI), especially by the Fe(II) present, causing the chromate concentration found to be lower than the original one.

Thomsen and Stern [3] described a method for determination of chromates with different solubility with sample leaching at high pH using 7% Na_2CO_3 . Alkaline leaching caused no significant reduction of Cr(VI). Cr(VI) was finally determined by FAAS and compared with results obtained by DPC spectrophotometry.

A method for collecting the welding fumes on air filters which were subsequently extracted with aqueous 3% Na_2CO_3 and 2% NaOH has been reported by Brescianni *et al.* [61]. The Cr(VI) was separated from the extract by the liquid ion exchanger Amberlite LA-2 before analysis by ETAAS. The separation also removes interferences from *e.g.* Ca, Fe, K and Na.

Airborne particulate-bound Cr(VI) has been determined by Ehman *et al.* [62]. The method uses a mildly alkaline extract of the particulates (aqueous solution of 0.005 % ethylenediamine) followed by removal of Cr(VI) on a Dowex 1X8-100 anion exchange resin. Chromium was detected by ICP-AES. The method was used to determine the reduction rate of Cr(VI) on a glass fibre filter.

Naranjit *et al.* [63] describe a method using the anion exchange resin (Anga 316) and the cation exchange resin (Dowex 50W-8X) for the separation of Cr(VI) and Cr(III) respectively. Aqueous extracts (buffer solutions of pH 3-5) of welding fumes were filtered through membrane filters, submitted to chromatography, and analyzed by FAAS.

X-ray fluorescence (XRF) allows the measurement of the total chromium concentration on filters in a non destructive way and with a minimum of sample handling. Arber *et al.* [64] attempted to extend the application to determine the Cr(VI) species. They studied the K-X ray emission lines of chromium in several oxidation states, and changes in peak position and profile were observed. These changes were then related to oxidation state and ligand environment, which enabled the Cr(VI) to total chromium ratio to be determined by XRF. The Cr K β' to Cr K $\beta_{1,3}$ intensity ratio was chosen as the most appropriate parameter. The method was applied to the determination of the Cr(VI) content of welding fume samples.

A similar approach was followed by Terada *et al.* [65] who collected steel welding fumes and pressed them into a 40 mm diameter disc for analysis by high resolution XRF. The ratio of Cr(III) to Cr(VI) was determined by examination of the Cr K α peak profile.

The ability of human blood erythrocytes to take up dissolved Cr(VI) in the presence of Cr(III) was exploited by Neidhart *et al.* [66] for speciation studies in, for instance, airborne particulates. The erythrocytes were immobilized in Ca alginate beads increasing mechanical stability during sampling of chromium. After sampling, the red blood cells were separated from the gel and subjected to a multiple step clean up procedure. The chromium content was determined by ETAAS with an O_2 ashing step in the furnace program to improve the ashing of the erythrocytes.

8.4 Cr speciation in biological samples

Speciation of trace elements in body fluids and tissues consist of defining the many biocomponents to which trace elements are bound and to quantify the element in relation to those particular molecules. This helps to explain their mobility, storage, retention and toxicity. Even more care has to be taken than for environmental samples to maintain the integrity of the often very labile metal-ligand association and to check the mass balance of the protein and the trace element throughout the isolation steps. A major problem is caused by the immense hazards of contamination or losses of the element, especially with the very low concentrations of chromium encountered in biological materials. The "true" serum chromium content for instance is now agreed to be 0.1 to 0.2 ng.ml⁻¹ [67]. An overview of techniques often used for speciation purposes in biological materials is given by Cornelis [68,69]. Only very few applications of speciation studies of chromium in biological materials have been successful.

Borguet *et al.* [70-72] performed a speciation study of chromium in plasma of continuous ambulatory peritoneal dialysis (CAPD) patients. The outcome of the work is given visually in Figure 1 [73].

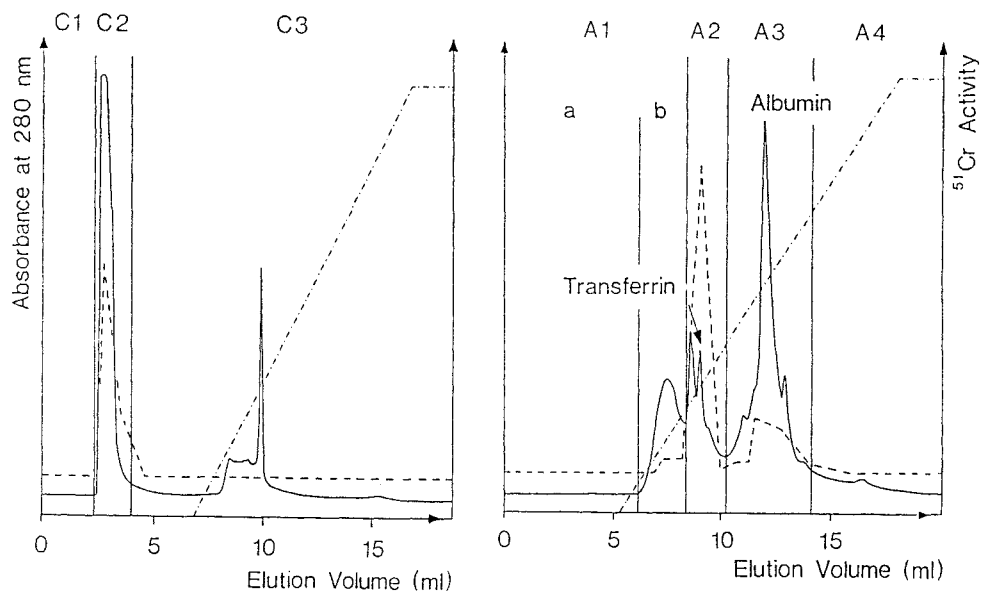


Figure 1: Chromatograms obtained with FPLC:liquid chromatography of:
a: cation exchange of ⁵¹Cr incubated plasma
b: anion exchange of fraction C2
(-) protein profiles, (---) ⁵¹Cr elution patterns, (-.-) salt gradient (0-0.5 mol.l⁻¹ NaCl)

The work was initiated by the finding of extremely high chromium levels in the serum of those patients (4.25 ng.ml^{-1}). They intended to identify the proteins to which chromium, originating from the dialysate, is bound and to quantify the chromium distributed over those proteins. Chromium is present as Cr(III) both in plasma and the dialysate. According to studies by Hopkins and Schwartz [74] and by Wallaey [75] it is mainly bound to albumin and transferrin in plasma of healthy individuals. The separation was performed with Fast Protein Liquid Chromatography (FPLC). This offers the advantage of a faster separation which reduces the time for interactions between the gels and the proteins and/or the chromium and thus prevents changes in the original chromium-protein association. The separation scheme consisted of a cation exchange MonoS column to remove disturbing agents. Unbound proteins were washed out with 0.025 mol.l^{-1} Tris.HCl pH 8 and were then chromatographed on a MonoQ anion exchanger. The absorbed proteins were eluted with a linear NaCl gradient ($0\text{--}0.5 \text{ mol.l}^{-1}$) in the same buffer. The proteins were followed by their UV absorption at 280 nm. Transferrin and albumin were quantified by nephelometry. Chromium could be traced by working with ^{51}Cr labelled plasma.

8.5 Use and availability of reference materials

No analytical method can be trusted to produce reliable results unless its accuracy has been controlled and documented by the use of a representative certified reference material. So far no useful reference materials are available for Cr(III) and Cr(VI) analysis in water and welding fumes.

In the past the NIST multielement water standards NBS SRM 1643 a and NBS SRM 1643 b, and the National Research Council of Canada river water standard NRCC SLRS-1 were used to check the total chromium recovery and give an idea about the performance of the method. Recently, NIST presented two new reference materials for speciation of Cr(III) and Cr(VI), respectively NIST SRM 2108 and NIST SRM 2109. The concentration of Cr(III) (1 g.l^{-1} in 1 % HCl solution) and Cr(VI) (1 g.l^{-1} in aqueous solution) are too high to be realistic, whereas the matrix is not representative for problems encountered in routine use.

Only the Proficiency Analytical Testing program conducted by the NIOSH includes chromium on filters. The quality control materials used however do not have a similar matrix as filters loaded with welding fumes. To date no useful reference material for filters loaded with welding fumes is available.

The European Commission has launched a project to develop a water and a filter (loaded with welding dust) reference material. The Measurements and Testing Programme (BCR) entrusted this task to the Laboratory of Analytical Chemistry, University Gent, Belgium, in cooperation with the Institute for Occupational Health, Copenhagen, Denmark.

When considering the production of a certified reference material some recommendations should be taken into account:

- The concentration of the element and the different forms of the element should be representative of the concentrations encountered in real world samples.
- The matrix should be similar to that of natural samples.

The species in the material should be stable for at least a few months; no exchange from one species to the other should occur, *e.g.* reduction of Cr(VI) to Cr(III).

Two water reference materials were envisaged; a material "A" representative for natural or drinking water (concentration range Cr(III)-Cr(VI) 10-40 $\mu\text{g.l}^{-1}$) and another one "B" typical for Cr(VI) determinations in leaching solutions of filters loaded with welding dust (concentration range Cr(VI) 5-10 mg.l^{-1}). Studies were carried out to investigate the stability of Cr(III) and Cr(VI) as a function of time, temperature, container material and solution. A $\text{HCO}_3^-/\text{H}_2\text{CO}_3$ (50 mmol.l^{-1}) buffer solution at pH 6.4 under a CO_2 blanket was most suitable to stabilize Cr(III) and Cr(VI) in solution A. In addition a matrix very close to that of real water was achieved. Solution B was stabilized in a carbonate buffer at pH 9.6. The work is described in detail by Dyg *et al.* [53,76]. Results of the stability tests are shown in Figure 2 [73].

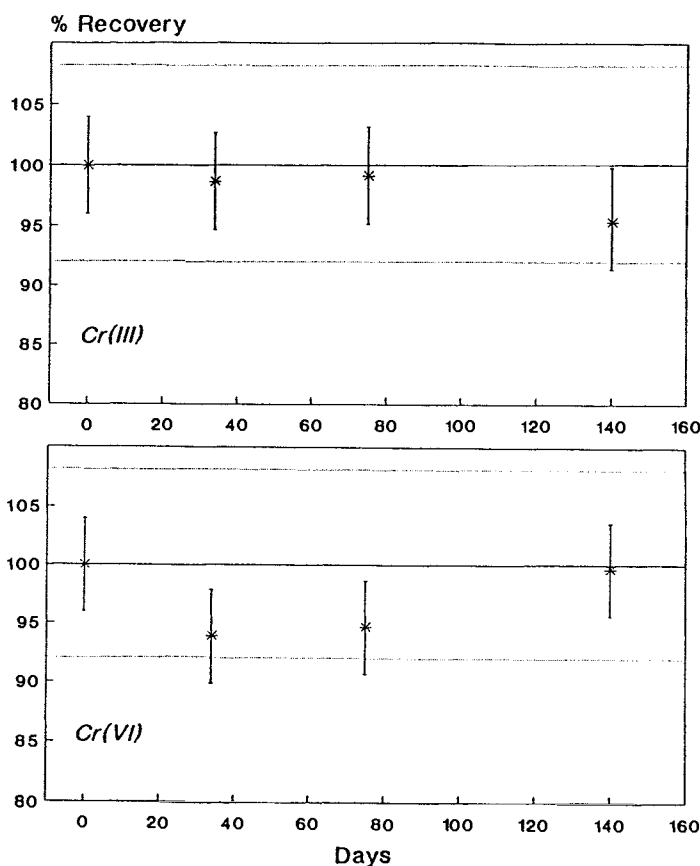


Figure 2: The recoveries of Cr(III) and Cr(VI) at a concentration of 25 $\mu\text{g.l}^{-1}$ in PTFE containers at 5 °C in a 50 mmol.l^{-1} $\text{HCO}_3^-/\text{H}_2\text{CO}_3$ buffer at pH 6.4 under a CO_2 blanket.

Because of its presumably unlimited shelflife, a freeze-dried product could be more interesting as a certified reference material. Therefore the work evolved in that direction. The recovery and parameters likely to influence the composition of the materials were investigated using ^{51}Cr labelled Cr(III) and Cr(VI). The materials were subjected to an intercomparison to gauge the present state of the art.

Similarly, a filter reference material loaded with known amounts of Cr(VI) was developed. One hundred filters were loaded with welding fume dust using a Sputnic air sampling unit containing 100 binderfree glass fibre filters. Collection was carried out in industrial working conditions using manual metal arc welding under well defined conditions. The filter load was carefully determined by weighing before and after exposure. The amount of Cr(VI) on the filters (around $100\text{ }\mu\text{g}$ per filter) corresponds to that usually encountered in occupational analyses. Finally the filters are stored in an inert atmosphere. The homogeneity and stability with regard to Cr(VI) were verified. For the homogeneity test, sixteen filters from different places in the Sputnic sampler were analyzed and proved the sampling to be adequately homogeneous. The stability with regard to Cr(VI) and total soluble chromium were verified after 1, 4 and 13 weeks storage at $-20\text{ }^{\circ}\text{C}$ and $+25\text{ }^{\circ}\text{C}$. No Cr(III) could be detected while the Cr(VI) concentrations remained unaltered. More information about the work is described by Dyg *et al.* [59].

These preliminary experiments proved the feasibility of producing a stable and homogeneous candidate reference material for both water and welding fumes. The work is now being continued and an intercomparison with several participants using different techniques is organized.

8.6 Future developments

The methods described above allow analysis of rather simple samples for which no digestion and little or no pretreatment is necessary. Solid and biological samples require more difficult pretreatment procedures where extreme care must be taken to preserve the original species in the sample. Chemical digestion procedures are usually not applicable. The application of enzymatic digestion to the preparation of tissues for chromium speciation determinations should be further investigated [9]. Also supercritical fluid extraction might be a promising separation technique in view of avoiding interconversion of species. The extractions can be performed at low temperatures and in a very short time thus being "safer" for easily oxidized or thermally labile compounds. Vela *et al.* [28] give a list of newer and alternative separation methods including capillary zone electrophoresis, countercurrent chromatography *etc.* that allow for a separation based on other physical or chemical properties than the ones used so far.

The colloidal or organically bound fractions of Cr(III) are not easily determined. It is important to be able to distinguish between bound Cr(III) and dissolved hydrolysed species of Cr(III) which are likely to be far more reactive. Future method development should address the problem of total chromium determination and the characterisation of the colloidal chromium fraction [44]. More research will also be necessary to distinguish between the different hexavalent salts including the insoluble, soluble and partially soluble salts.

Mugo and Orians [41] say that "Further investigations are clearly needed to provide a complete understanding of the geo chemical cycle of this element. In particular the in situ use of "natural" laboratories such as anoxic and seasonally anoxic basins to monitor Cr(III)/Cr(VI) interconversion as a result of changes in oxygen concentration, coupled with kinetic and speciation studies in the laboratory will be important."

Vela *et al.* [28] regret the lack of suitable reference materials: "To date most emphasis in trace elemental speciation has been placed on development of the actual separation techniques. This is only a portion of the total procedure for determining species in a given sample. The optimization of these methods was initiated with the use of chemical standards, followed by validation using real samples including certified standard reference materials. One limitation to validation of these speciation methods is the availability of reference materials for certified concentrations. If a wider variety of these materials were available, more speciation techniques could be developed as approved methods for toxicological and environmental studies".

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9.

Determination of aluminium species in natural waters

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The use of aluminium by man is continuously growing and therefore living organisms have to contend with an ever increasing environmental exposure to elevated levels and to unnatural forms or species of this element [1]. The potential toxicity of aluminium has become a major medical and environmental issue. For a long time, aluminium was not considered to be toxic to human beings, but this attitude changed in the 1970's, when aluminium was first associated with dialysis dementia syndrome [2]. Since then, comparatively high aluminium levels in body tissues have been implicated in various clinical disorders suffered by patients with chronic renal failure undergoing regular haemodialysis, where, for various reasons, water and dialysis fluids high in aluminium were used. These disorders include dialysis encephalopathy, dialysis osteodystrophy, parathyroid dysfunction and microcytic anaemia [3,4] and, perhaps most controversial, Alzheimer's Disease (AD) [5]. Epidemiological studies seem to demonstrate a positive link between occurrence of AD and mean aluminium levels in drinking water [6,7]. This is somewhat disturbing as alum (aluminium sulphate) is widely used as a coagulant in the water industry. Already several accidents such as the one at Camelford, Cornwall, U.K., in 1988, have occurred [8]. In that incident, too much alum was added to the local water supply and the population exhibited many of the symptoms of acute short term aluminium poisoning (although the long term effects of such an event will only become clear over time).

From an environmental standpoint, the recognition that "acid rain" mobilizes aluminium from poorly buffered soils into the aquatic environment has increased the awareness and concern about aluminium toxicity to aquatic organisms. The acidification of fresh water lakes and rivers in the U.S.A., Canada, and particularly the Scandinavian countries, and the subsequent rise in dissolved aluminium levels, has been linked to the decline in fish numbers, and in some cases, to the total elimination of entire fish populations [9-12]. Concern in this area has particularly been focused upon storm and snow-melt episodes,

which can provide a surge of pollutants, including aluminium, and can cause extremely toxic short term conditions [13-18].

Aluminium has also been shown to stunt the growth of certain fresh water algae at pH 5.8-6.2, and at very low concentrations ($<5 \mu\text{g l}^{-1}$) [19], and to inhibit plant growth in acidic soils [20]. Commercial crop plants that are susceptible to the toxic effects of aluminium include cotton [21], barley [22], and wheat [23]. The toxic effects of this element in plants usually manifest themselves as poor root development with a subsequent decline in crop yields.

9.1 The toxic aluminium species in aquatic systems

In aquatic systems, it appears that dissolved aluminium is toxic to fish. Several workers [8,9] have demonstrated that the $\text{Al}(\text{OH})_2^+$ species seems to be the most toxic to fish. In addition, other workers [24], using thermodynamic calculations in conjunction with fish toxicological experiments, have pointed to the $\text{Al}(\text{OH})^{2+}$ species as also toxic to fish. For brown trout, $20 \mu\text{g l}^{-1}$ of total aluminium at pH 4.4-5.2 stunted fish growth while levels of $50 \mu\text{g l}^{-1}$ or more induced high mortality rates. However, the picture is rather more complicated than this as different species of fish seem to exhibit different toxicological responses to different aluminium concentrations and water pH's. In other words some species are more susceptible to aluminium intoxication than others and this susceptibility depends upon their different life stages [11]. In one report, under acid conditions (pH 4.2-4.8), aluminium improved egg survival but increased the mortality rate of brook trout sac fry [25]. The mechanism of aluminium toxicity towards fish has been attributed to two main factors [24,26-28]. Firstly, at higher aluminium concentrations ($\approx 0.5 \text{ mg l}^{-1}$) and adequate pH, colloidal aluminium hydroxide species form which could clog the gills of the fish, thereby inhibiting proper respiratory function. Secondly, at more modest aluminium levels ($< 0.2 \text{ mg l}^{-1}$), in waters with a low calcium content, aluminium alters the passive permeability to ions of the epithelium cells in the fish gills, disrupting normal osmoregulatory balance, and lowering plasma concentrations of Na^+ and Cl^- ions [26].

9.2 Analytical methods for the speciation of aluminium

As with many elements, total aluminium determinations are of limited value for environmental toxicological purposes because it is the bioavailability of an element which governs its toxicity and this bioavailability is, in turn, dictated by its physicochemical form (metal speciation). Aluminium is no exception to this rule. As mentioned above, in natural acidic waters the aluminium species most toxic to fish are the $\text{Al}(\text{OH})_2^+$ and $\text{Al}(\text{OH})^{2+}$ species. No specific, direct method for the separation and individual quantification of these particular species exists today. However, a number of fractionation procedures have been developed over the last 15 years in order to distinguish between the various aqueous forms of aluminium. These methods include dialysis, ion-exchange (both batch and column), HPLC, F ion-selective electrode, NMR, species specific extractions, filtration and computational techniques. All of these procedures usually measure operationally defined aluminium fractions (*i.e.* groups of aluminium species are measured, rather than a species, and the values obtained depend upon the precise procedure used for the analysis). Therefore, present speciation procedures may measure slightly different forms of aluminium depending on the conditions, and thus they should accordingly provide different results.

Here the most frequently used methods and techniques for aluminium speciation analysis in waters are summarized, together with their common ground and the grey areas which need to be clarified if aluminium speciation analysis in waters is to become a well defined and regular determination to evaluate the potential aluminium toxicity of waters to plants, fish and humans.

9.2.1 8-Hydroxyquinoline extraction procedures

Various methods have evolved based on the complexation of monomeric aluminium species with 8-hydroxyquinoline (oxine) and the rapid extraction of the formed Al(oxinate)_3 complex into organic solvents. Some of the more basic aspects of these methods were investigated by Okura *et al.* in the 1960's [29]. They used chloroform as the solvent and determined the aluminium content from the absorbance of the Al(oxinate)_3 in the organic extract at 390-420 nm. For their experimental conditions, the extracted aluminium had a degree of polymerization close to 1 (i.e. monomeric). Other workers showed that by allowing the complexation reaction to proceed for up to 6 h, an estimate of the polymeric aluminium content could also be obtained [30].

This method was later modified by Barnes [31], who, after filtering the sample through 0.1 or 0.45 μm filters, added the oxine, adjusted the pH to 8.3, and extracted the resulting Al(oxinate)_3 complex into methyl isobutyl ketone (MIBK). Detection of the aluminium content was finally accomplished by atomic absorption spectroscopy.

May *et al.* [32] used a toluene extraction at pH 5.0, but subsequently found that the extraction at pH 8.3 eliminated F^- interferences. LaZerte [15], further developed this method by employing equilibrium dialysis as a pretreatment of the samples, prior to the oxine extraction step. He used a 1000 Da nominal cutoff dialysis tubing, and extracted the inorganic monomeric aluminium over a 24 h period at 20-25 °C. He concluded that this would extract 90-95% of the monomeric inorganic aluminium, with minimal diffusion of the aluminium fulvic acid complexes that can be found in natural waters. Electrothermal Atomic Absorption Spectrometry (ETAAS) was used for the detection of the aluminium in the fractions. A schematic representation of the aluminium fraction obtainable using this method is given in Figure 1.

This oxine extraction method has been automated very recently using a continuous chloroform extraction of the Al(oxinate)_3 complex at pH 5.0, with ultraviolet (UV) spectrophotometric detection [33]. This last method differs slightly from the previous ones as the authors claim that the AlF^{2+} species is not included in their "quickly reacting" aluminium fraction because they use a 2-3 s complexation time (as shown in Figure 1, 15 s are normally used).

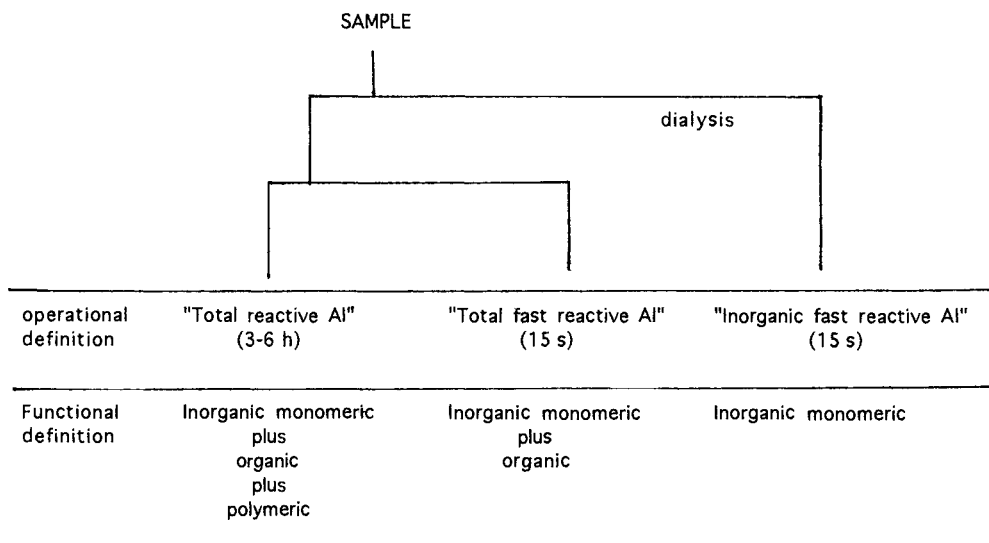


Figure 1: Definitions of aluminium species as obtained by the dialysis/8-hydroxyquinoline/MIBK speciation procedure (modified from [15])

9.2.2 Driscoll methods

The so-called Driscoll methods are all based around an ion-exchange separation of the monomeric inorganic aluminium from the monomeric organic aluminium. The column packing is Amberlite IR-120 cation exchange resin. One of the first reports on the use of this particular resin for the speciation of aluminium was by Okura et al. [29]. They expressed the concern that the ion-exchange resin released H^+ during the exchange process, thus changing the equilibrium between mono- and polymeric species. However, they concluded that the depolymerization of the polymeric forms of aluminium is quite slow at low acidities, and that any speciation change during the ion-exchange process would be negligible. We have called all the methods presented in this section "Driscoll" methods, because Driscoll et al. [9] were the first to publish a complete aluminium fractionation technique based around this cation exchange resin. In essence, the Driscoll method directly measures three operationally defined fractions:

- acid reactive aluminium (Al_r)
- total monomeric aluminium (Al_m)
- non-labile monomeric aluminium (Al_n)

An attempt to clarify the different concepts and definitions of the Driscoll fractionation procedure is shown in Figure 2.

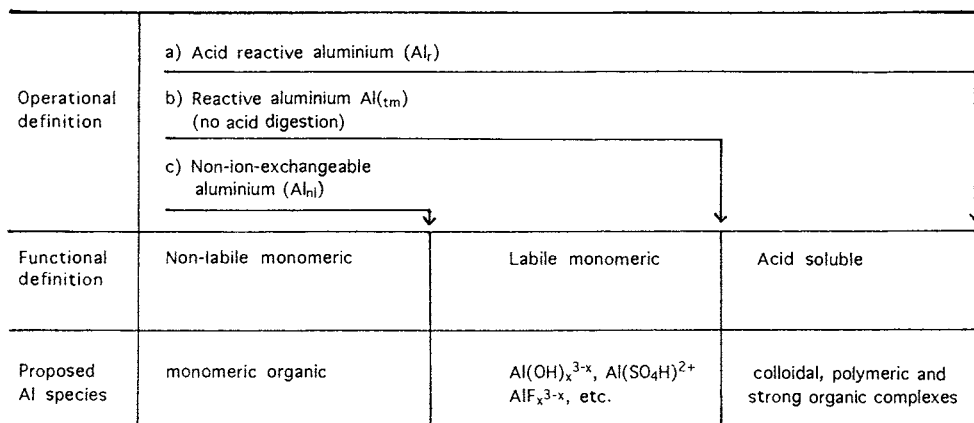


Figure 2: Schematic representation of the Driscoll method for the fractionation of aluminium in waters (modified from [14])

The first published methods used a colorimetric 8-hydroxy-7-iodoquinoline-5-sulphonic acid (ferron)/ orthophenanthroline method for the complexation of the aluminium in the desired fractions [9,14]. The Al_r fraction was determined after acidification of the sample for 30 min before the final colorimetric determination. The Al_{tm} was determined through direct complexation with ferron, without acidification. The Al_{ni} (or monomeric organic) fraction was taken as the fraction determined by ferron without acidification, after the sample had been passed through the ion-exchange column. From these three experimentally obtained fractions, two more could be calculated: "acid soluble" aluminium is taken as the difference between Al_r and Al_{tm} and the "labile monomeric" aluminium fraction, considered the more toxic one, Al_{lm} is taken as the difference between the Al_{tm} and Al_{ni} fractions. In this fractionation scheme the Al_{lm} fraction is thought to include free hydrated Al^{3+} along with its possible fluoride, sulphate and hydroxy complexes. Modifications of the Driscoll method include the use of the Barnes oxine/MIBK extraction [31,34], and probably the most popular variation in use at the present time, the technique using pyrocatechol violet detection of the "Driscoll" aluminium fractions [34-37].

We have summarized some of the more common modifications of the "Driscoll" methods proposed, together with experimental details, in Table 1.

Table 1: Experimental details of some of the Driscoll fractionation procedures for aluminium in natural waters

Reagent	Reaction Time ($Al_{in} + Al_{in}$)	Method	Acid Attack Time (Al_t)	Column Size	Detection Method	DL $\mu g\ l^{-1}$	REF
Ferron	instant	batch	30 min	----	UV-Vis	----	9,16
Ferron	-----	batch	30 min	----	UV-Vis	----	35
Ferron/ MIBK	instant	batch	pH 1.5 1 h	9.5 cm ³ volume	370 nm ETA-AAS	----	14
Ferron/ MIBK	instant	batch	pH 1.0 1 h	----	ETA-AAS	----	34
PVC	4 min	batch	pH 1.0 395 nm	----	UV-Vis	----	35
PCV	4 min	batch	34 h pH 1.4	----	581 nm	----	34
PCV	10 min	batch	1 h pH 1.0	----	UV-Vis	----	36
PCV	10 min	batch	----	0.5x5.0cm	581 nm	----	37
PCV	10 min	batch	O.1 N HCL	----	UV-Vis	5	38
PCV	10 min	batch	1 h pH 1.0	0.4x7.0cm	UV-Vis	6	39
PCV	≈4 min	FIA	----	0.3x13 cm	UV-Vis	----	40
PCV	4-5 min	FIA	----	0.3x7.0cm	UV-Vis	10	38
8-HQS	15 s	FIA	----	0.3x14 cm	580 nm	0.9	
				0.3x5.0cm	Fluorescence		

From the data available, there is to no consensus as to the precise experimental conditions to be used. This is especially true where the analytical separation is concerned. The resin in the column has to be conditioned with a solution of NaCl having the same ionic strength and pH as the water samples to be analyzed and this is one of the main drawbacks of the method because the solution of NaCl has to be passed through the column until the pH of the effluent does not change more than ± 0.2 pH units. To cut down on column conditioning time, some workers have found that conditioning the column from the Na^+ form, not from the recommended H^+ form as in the original Driscoll protocol [9], is an effective approach to shorten this preparative step [38]. Other useful modifications for the Driscoll/PCV method include a 10 min PCV reaction time [37], small (3.5 ml) samples [36], a 1 cm^3 column of Amberlite IR-120 [37], and a split calibration curve (0-100 and 100-400 $\mu\text{g l}^{-1}$) [39]. A full Driscoll/PCV speciation procedure including all these modifications has been published elsewhere [38].

Some authors claim that detection limits of 5 $\mu\text{g l}^{-1}$ are easily obtainable [37] by using the batch PCV detection method. However, it has been reported that daily DL's range between 5-10 $\mu\text{g l}^{-1}$ and that personnel who are highly acquainted with the method were needed to achieve DL's down to <10 $\mu\text{g l}^{-1}$ [38]. This poses a problem when the non-labile or "monomeric organic fraction" of aluminium to be measured is very small. Errors for Al_m can become high, and, because the Driscoll method is a subtraction method, the uncertainty of the calculated Al_m fraction ($\text{Al}_m - \text{Al}_n$) can become unacceptable.

To increase the precision, sample throughput, and practicality of the Driscoll method, several Flow Injection Analysis (FIA) systems have been designed both for the PCV method [39,40] and for the detection with a fluorimetric determination of the 8-hydroxyquinoline-5-sulphonic acid (8-HQS) aluminium complex in a micellar medium (cetyltrimethylammonium bromide (CTAB)) [38,41]. The PCV automated system of LaZerte et al. [39] is slightly different from the other PCV systems (both batch and FIA) in that it seems to measure the "PCV reactive aluminium fraction", analogous to Driscoll's "labile monomeric fraction". The main advantages of these FIA systems (the 8-HQS system is shown in Figure 3) are that they utilize smaller analytical columns (thus reducing conditioning problems), and smaller samples (μl 's). Therefore they are more rapid and usually more precise [38].

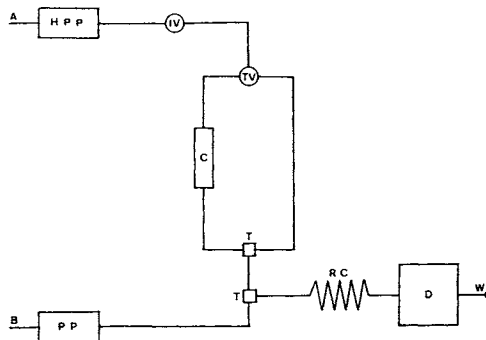


Figure 3: Schematic diagram of the 8-hydroxyquinoline-5-sulphonic acid speciation method [38]. HHP= high pressure pump, IV= injection valve, TW= two-way valve, C= ion exchange column, D= fluorimetric detector, PP= peristaltic pump, W= waste, RC= reaction coil, T= T-piece, A= NaCl mobile phase and B= post-column reagents

One important concern about the Driscoll method is which species are actually retained by the column: that is, the true chemical nature of the Al_m fraction, considered the most toxic. Some authors have shown that the retention characteristics observed are sample flow dependent [14,42], and that each column needs to be individually characterized for aluminium retention. Also, questions have been raised as to whether the use of short reaction times with complexation reagents (PCV, oxine etc.) is a good indication of monomeric aluminium. In fact Morrison [43] has suggested that the measurement obtained with short reaction times is a reflection of colloidal reactive species concentration rather than the monomeric inorganic aluminium content, especially in the case of PCV.

9.2.3 Chelex-100 based methods

Several procedures using Chelex-100 (a styrene/divinylbenzene co-polymer with iminodiacetate functional groups) have also been developed. For batch systems, where conditioned resin is stirred with the water sample for up to 4 h, the "labile" aluminium fraction is calculated from the difference between the total aluminium concentration in the sample found before and after ion-exchange has taken place. The resin has to be very carefully preconditioned in a nitric acidified solution (pH 5.0), $Ca(NO_3)_2$ and $MgSO_4$ (at concentrations normally found in natural waters) [44,45]. Campbell et al. [45] used comparatively short reaction times of 30-60 min, in batch mode, to distinguish readily exchangeable species from other forms of aluminium. Miller and Andelman [46,47] modified this method slightly, and using reaction times of 1, 4, and 24 h, fractionated aluminium into the four categories shown in Table 2. The main problem with using this type of technique for the determination of the toxic aluminium species in water is that it groups together the monomeric hydroxy, fluoro-aluminium and low molecular weight (LMW) polymeric species in the same fraction (> 85% of these species exchange under 30 min [45]). Moreover, the operational definitions do not match adequately those of the more popular "Driscoll" methods (compare Table 2 with Figure 2).

Further developments using Chelex-100 include a most recent, exhaustive, but rather complicated fractionation scheme, using a batch (unconditioned) Chelex-100 procedure combined with Ultrafiltration [48]. Another recent and most interesting method, using a Chelex-100 column procedure, claims to be able to selectively elute the AlF^{2+} species from the other retained "labile" monomeric species using different strength elutents containing HCl [49]. However, the separation of the AlF^{2+} species from the other inorganic monomeric aluminium species was not yet quantitative.

Table 2: Aluminium fractionation scheme according to the batch Chelex-100 method of Miller and Andelman [47]

Operational definition	Functional definition
Rapidly exchangeable (1 h reaction)	Monomeric and small polymeric cationically charged species
Moderately fast exchangeable (4 h reaction)	Mainly inorganic aluminium complexes
Slowly exchangeable (24 h reaction)	Mainly organically complexed forms of aluminium
Non-exchangeable	Strong alumino-organic complexes, colloidal or crystalline forms

9.2.4 Fluoride electrode methods

These are indirect methods based around computational procedures, using estimates of the monomeric inorganic aluminium fraction and "free" and "total" fluoride concentrations. Normally, "free" fluoride is determined by direct measurement with an ion-selective electrode. The "total" fluoride concentration is determined using the same ion-selective electrode technique, but after the addition of a total ionic strength buffer (TISAB). LaZerte [15] has reviewed the main fluoride electrode methods for aluminium speciation. When an estimate of the inorganic monomeric aluminium is available, "free" fluoride analytical data can be used to calculate Al^{3+} activities and the proportions of the other monomeric aluminium species. However, the concentrations of other possible binding ligands existing in the water have to be known as well. Driscoll [14] has described a method with fluoride electrodes which eliminates the necessity of a direct measurement of the inorganic monomeric aluminium fraction. However, these methods should only be employed for waters with a high aluminium content and a $\text{pH} < 5.5$, as they need a large amount of inorganic monomeric aluminium to ensure that $\text{AlF}_x^{(3-x)+}$ complexes make up a large proportion of the total fluoride present in the sample. Finally, "free" fluoride measurements in natural waters can be very slow and prone to error, particularly in the presence of other fluoride complexing cations (e.g. Fe^{3+}).

9.2.5 HPLC Methods

In principle, HPLC, with its more powerful separation abilities, should be a most useful speciation tool. Bertsch and Anderson [42] have described a system using a Dionex CG3 column with a NH_4Cl solution as the mobile phase at a pH range of 2.0-4.2.

Aluminium was detected by post-column derivatization with 4,5-dihydroxybenzene-disulphonic acid (Tiron) at pH 6.3. They managed to separate the $\text{Al}(\text{H}_2\text{O})_6^{3+}$ from two tentatively identified aluminium fluoride species (AlF^{2+} and AlF_2^+), and to quantify the monomeric aluminium fraction. They also reported some disadvantages regarding eluent ionic strengths and that pH seriously influenced the observed speciation of aluminium in the presence of F⁻ and citrate ligands. Jones [50] has reported the use of a Dionex CG2 guard column maintained at 50 °C in a water bath for aluminium speciation. He used a potassium sulphate (pH 3.0) mobile phase with post-column derivatization of the aluminium with 8-HQS at pH 4.1. Fluorescence was measured at 512 nm (excitation at 360 nm) [51]. He was able to quantify the $\text{Al}(\text{OH})_x^{(3-x)+}$ fraction with this system, excluding the fluoride and organically bound aluminium species. A typical chromatographic separation of the AlF^{2+} species from the total inorganic aluminium in a synthetic solution using the system of Jones [50] is shown in Figure 4. Both Bertsch [42] and Jones [50] claim that the slower reacting polymeric species (e.g. $\text{Al}_2(\text{OH})_2^{4+}$) would be strongly retained on their ion-exchange columns and thus would not interfere with the determination of the mononuclear aluminium species. The major point of these HPLC methods is that they separate the aluminium fluoride species from the other inorganic monomeric aluminium species (classic "Driscoll" methods do not).

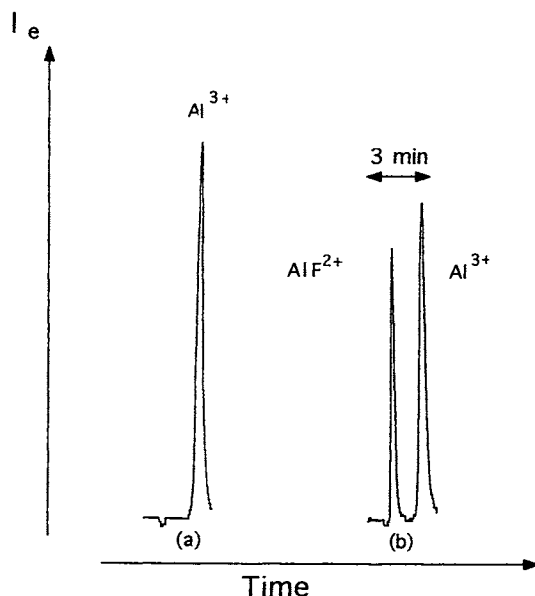


Figure 4: Typical separation of Al^{3+} and AlF^{2+} species using the HPLC system of Jones [50].

a) 18 $\mu\text{mol.l}^{-1}$ aluminium solution

b) 18 $\mu\text{mol.l}^{-1}$ aluminium plus 2.5 $\mu\text{mol.l}^{-1}$ fluoride (as NaF)

9.2.6 ^{27}Al NMR Studies

NMR techniques have been utilized by Bertsch *et al.* [42,52] for the determination of Al^{3+} concentrations and for other $\text{Al}(\text{OH})_x^{(3-x)+}$ species. Problems with maintaining identical instrumental conditions, differences between acidified and unacidified solutions and low sensitivity are the main drawbacks. However, good agreement with values obtained by the HPLC technique for the uncoupled $\text{Al}(\text{H}_2\text{O})_6^{3+}$ species were obtained [42]. The authors concluded that their method may be particularly useful for aluminium speciation in synthetic solutions, *i.e.* as utilized in kinetic and fish toxicological investigations. This has indeed been the case with reports of the use of ^{27}Al NMR to study the binding and stability of a variety of aluminium complexes such as acetate and oxalate [53] as well as citrate [54].

9.3 Water sampling for aluminium speciation analysis

Conventional linear polyethylene bottles are the most commonly used containers for the sampling and storage of water samples [55]. A comprehensive review on container cleaning methods [56], in which 13 published cleaning methods were investigated and compared, concluded that a 48 h soak with 10 % v/v HNO_3 was adequate for both the preliminary cleaning of new polythene bottles, and for the removal of trace metal contamination. Containers treated in this way were found to be suitable for the storage of river and tap waters for up to 28 days [56]. Unfortunately, aluminium was not included in their tests. Fairman *et al.* [57] have carried out tests exclusively for aluminium and high density polyethylene containers along with a cleaning procedure of:

- rinsing with distilled water to remove solid particles.
- leaching with 10 % v/v HNO_3 for 48 h.
- rinsing with Milli-Q (18 megohms or equivalent) water.
- filling with ultra pure water until use.

In this later study, it was also found that trace concentrations of aluminium ($< 50 \mu\text{g l}^{-1}$) were stable in high density polyethylene containers when stored in 0.1 mol.l $^{-1}$ HNO_3 or 0.1 mol.l $^{-1}$ citric acid media. No leaching or adsorption losses were observed. Conversely, for polypropylene and polystyrene materials it was observed that aluminium was leached from the container walls after 30 days [57].

As discussed by Florence [55], most discrepancies over trace metal losses to polyethylene containers occur between experiments where synthetic solutions were used and those which used natural waters. The natural waters normally contain solubilizing ligands present, which prevent any adsorptive loss of trace metals to the container wall. Fairman *et al.* [57] stored natural lake waters for up to one year in precleaned polyethylene containers and found that after 30 days or so, losses were observed in the total aluminium concentration (Figure 5). These losses, however, coincided with an observed rise in sample pH, (probably due to the degassing of CO_2), and the losses could be explained by the precipitation of polymeric aluminium hydroxy species rather than adsorption of aluminium by the container material.

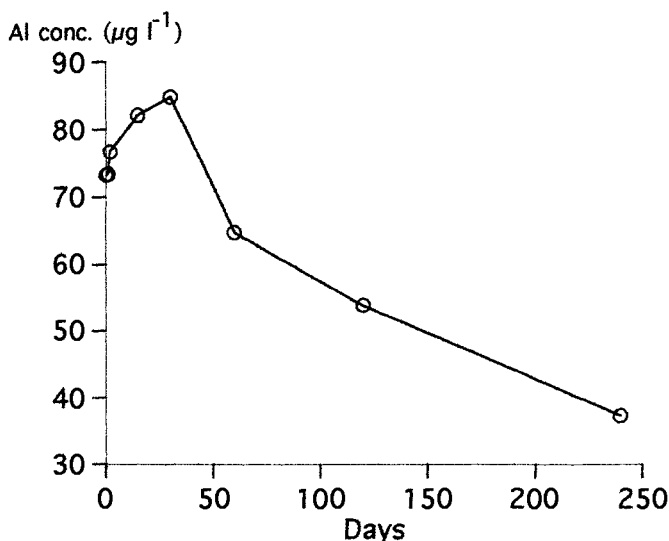


Figure 5: Loss of observed total aluminium from tap water stored at ambient temperature in pre-cleaned polyethylene containers (Analysis by ETAAS)

One further study [58], which combined hollow-fibre ultrafiltration with the Barnes-Driscoll method, concluded that the equilibrium processes between dissolved aluminium and meta-stable aluminium hydroxy species is fairly rapid and temperature sensitive. These authors concluded that, at best, aluminium speciation analysis should be performed immediately and, if samples are to be stored for any length of time, then the colloidal and particulate matter should be removed and the original temperature of the sample should be maintained.

9.3.1 To filter or not to filter

A close examination of all the speciation schemes described above shows that the question of sample filtration is one of the most controversial among the various methodologies and individual research groups dealing with the storage and sampling of waters earmarked for aluminium speciation analysis. Part of the problem stems from the fact that different workers have been applying the methods to a wide variety of scientific targets *e.g.* indicators of aluminium toxicity, soil chemistry and acid rain mobilization of labile aluminium species to name but a few.

For example, as said earlier [9,24], the toxic aluminium species to fish have been identified as the $\text{Al}(\text{OH})^{2+}$ and $\text{Al}(\text{OH})_2^+$ species. These species would be contained in the dialysis fast reactive fraction (Figure 1), or in the inorganic monomeric fraction (Figure 2) or in the rapidly exchangeable fraction (Table 2), depending on which speciation scheme you chose to use. However, the question is: will the distribution of

species in these operationally defined aluminium fractions change upon filtration? It has been suggested that "dissolved metal" is the fraction that passes through a $0.45\ \mu\text{m}$ filter [45,55]. However, many workers use a $0.15\ \mu\text{m}$ filter to distinguish between suspended and dissolved forms [31]. In fact some investigators do not filter at all, using eye judgement as to decide whether samples need it or not [35,39].

Goenaga *et al.* [59] have made a thorough study, trying to clarify this question for aluminium speciation. They state that aluminium adsorbed onto suspended solids is relatively labile and could account for a considerable proportion of the Al_{lm} fraction measured in water samples. Using the Driscoll/PCV method, they demonstrated that for a range of water samples containing various amounts of suspended solids, the Al_{lm} fraction decreased with decreasing filter pore size used, and that the decrease was greater in samples with higher suspended solids content. Because of this, they modified the Driscoll fractionation protocol as follows [59]: Al_{f} is now the amount directly detected by PCV in unfiltered samples, monomeric aluminium Al_{m} is as detected by PCV in filtered samples ($0.015\ \mu\text{m}$ pore size), Al_{n} as detected by PCV in the ion-exchange effluent of previously filtered samples. Therefore Al_{lm} is now taken as the difference between Al_{m} and Al_{n} [36]. According to the authors [59], this procedure minimizes the errors originating from labile aluminium adsorbed onto suspended solids in the determination of the inorganic monomeric aluminium fraction.

9.4 Validation of aluminium speciation analytical methods

Comparisons of different aluminium speciation methods are rather scarce. A selection of such exercises is given in Table 3. From these limited results it would seem that most of the aluminium speciation methods currently in use (particularly the Driscoll-based ones) uniformly assess the toxic aluminium fraction in acidic waters. However, in all of these comparisons, the compared methods were both performed in the same laboratory, and presumably by the same analysts. It is well known in inter-method comparison circles that this type of validation approach nearly always produces good results.

Some laboratories have tried to either standardize or validate different methodologies by interlaboratory comparison exercises [34]. However, little or no data from these programs have found their way into the international scientific literature.

One interlaboratory method comparison exercise for aluminium speciation has been published [57]. Results obtained using a standard Driscoll/PCV method for the determination of the "labile monomeric" aluminium fraction were tested and compared to three other aluminium speciation procedures. Each of the three participant laboratories carried out both the standard Driscoll method, used as a reference method, and its own method applied to the same water samples. For certain aluminium contents in waters (ranging from $100\text{--}200\ \mu\text{g.l}^{-1}$), the standardized Driscoll/PCV method produced extremely good results (as summarized in Table 4), with pooled values for the Al_{lm} fraction having RSD's of 6-15%. It was concluded that for sufficiently stable aluminium levels in water the Driscoll/PCV method was portable, and could be used as the basis for a standardized aluminium speciation scheme. A program to control each participating laboratory's ability to analyse trace levels of total aluminium in water samples was also recommended [57].

Table 3: Summary of some published intermethod comparison exercises for aluminium speciation

Techniques	Comments	Ref.
Computational F- vs. Oxine/dialysis	At pH < 5.5, $R^2=0.82$ for inorganic monomeric aluminium	15
Dialysis vs. Driscoll/PCV	For inorganic monomeric aluminium $R^2=0.99$. Dialysis underestimates PCV values by 6 %.	39
Driscoll/Ferron vs. Driscoll/PCV	Al_f gave good and Al_m and Al_n gave satisfactory agreement. PCV method generally better.	34
Driscoll/oxine/ MIBK extraction vs. Driscoll/PCV	Systematic differences between Al_m and Al_n fractions. However, good agreement for Al_m , $R^2=0.99$.	35
Driscoll/PCV vs. Three alternative methods	Al_f and Al_m gave good agreement. Care has to be taken as one method (HPLC) did not include Al-fluoride species in the Al_m fraction.	57

Briefly, although the results presented in Table 3 appear quite positive and promising, aluminium speciation data is still too scarce and procedures have yet to be rigorously tested for waters in international inter-laboratory method comparison exercises for adequate validation of the proposed aluminium speciation schemes and methods to finally be achieved.

Table 4: Results for a Inter-laboratory method comparison exercise using a standardised batch Driscoll/PCV fractionation method against three other methods [57].

		Batch Driscoll/PCV Al_t	Al_{lm}	Alternative method Al_{lm}
Sample A				
Laboratory	1	58.1 ± 2.7	37.5 ± 2.5	46.7 ± 1.0
	2	62.6 ± 2.6	46.7 ± 4.3	48.3 ± 5.0
	3	57.1 ± 2.4	36.3 ± 4.2	52.1 ± 7.5
Sample B				
Laboratory	1	146 ± 5.4	84.4 ± 2.6	83.7 ± 6.1
	2	102 ± 3.5	84.5 ± 2.9	$62.1 \pm 11^*$
	3	113 ± 15	-----	81.1 ± 9.4

n=4

* excludes Al-fluoride species

9.5 Conclusions and future perspectives

With the recognition of the fact that the physicochemical form of an element dictates its bioavailability, toxicity, and environmental impact, speciation analysis has taken on a new importance within the analytical community. Adequate analytical technologies, able to tackle the problem of elemental speciation, are increasingly required in the agricultural, environmental and medical fields.

From an analytical standpoint, three categories of speciation analysis have been identified [60] which are characterized by an increase in the degree of difficulty of analysis, which in turn is related to the lability of the various species. These three categories are defined as:

- a) "Thermodynamically stable and kinetically inert" species (e.g. stable organometallic compounds such as tributyltin (TBT));

b) "Thermodynamically stable but kinetically active" species (e.g. different redox forms of an element able to rapidly react accordingly to environmental electrochemical potentials);

c) "weakly bound, kinetically active" compounds, a definition which would include operationally defined groups of species such as the "labile monomeric" aluminium fraction as defined by the Driscoll fractionation schemes.

In dealing with speciation problems, the analyst continuously has to bear in mind these definitions, as they may influence any part of the analytical protocols developed e.g. sampling, storage, pretreatments (preconcentration *etc.*) separation and measurement.

Aluminium speciation in waters has been shown to be virtually "operationally defined" at the present time. Final improvements in analytical techniques or in the quality control of such speciation determinations demand an answer to the basic question: what aluminium species do we need to analyze? A possible way forward would be the standardization of the analysis for the toxic aluminium species to fish e.g. $\text{Al}(\text{OH})^{2+}$, as an indication of water quality. But, who has proved conclusively that other inorganic species e.g. $\text{Al}(\text{OH})_2^+$, AlO_2^- , AlCl^{2+} , *etc.*, which quickly convert to each other, do not produce similar toxicity problems? [61]. Do we have at the present time the analytical methods to distinguish and measure reliably only a given species (e.g. $\text{Al}(\text{OH})^{2+}$ in real samples (water, biological fluids and tissues *etc.*)? While these questions are not properly answered, perhaps it is better to use the term "speciation" in a not so rigorous sense, describing a group of species instead of only one. We believe that "something is better than nothing", and therefore aluminium speciation analysis should be developed at least along two main paths: a) research on new bioanalytical techniques to answer the toxicity questions above, and b) refinement of the "operationally defined" present methods along with inter-laboratory method comparisons in order to obtain comparable results for the "toxic aluminium species" in waters.

In order to effectively pursue this latter objective, at least two practical actions can be envisaged:

(i) Establishment of a standard method of analysis: this would offer many advantages. One of the most obvious would be in the comparison and validation of new speciation techniques (which could be checked against the standard method). Moreover, water quality, relative to aluminium toxicity to fish, could be internationalized by accepting the value of the Al_m fraction provided by this standard method. However, it should be noted that more research is needed in order to finally establish such a "standard method". For example, if the Driscoll/PCV method was accepted as the standard, some problems such as the inclusion of the aluminium fluoride species in the toxic fraction would have to be looked at. Such a standard method of analysis would also have to include standardized sampling and sample storage protocols, orientated specially towards aluminium speciation analysis. Modest advances along these lines are probably the way forward while basic progress is being made towards evaluation of individual species toxicity, their analytical separation and final reliable determination.

(ii) Availability of reference waters with certified aluminium fractions: several reference waters already exist with certified total elemental content and for a variety of matrices (e.g. artificial freshwater [62]). However, certified trace metal species content in such reference waters is virtually non-existent at the present time. In the case of aluminium, the availability of such an important quality control tool is hindered by some important

problems in:

a) Certification: a clear definition of species or aluminium fractions on which to provide certification data is missing. In the case of aluminium it is obvious that at the present time the particular preconcentration/separation/detection methods chosen to provide the certification data will determine the aluminium species or fraction selected.

b) Formulation of unified sampling and sample storage procedures. These procedures or protocols are necessary if samples taken in the field are to be treated the same as any reference water in the laboratory, in order to preserve the integrity of the aluminium species present. The question of filtration, or otherwise, and with what size filter, is a key-point here. Maybe one approach would be the isolation of the desired species in the field (such techniques already exist for other elements [63]), mainly based around the retention of the desired species in mini-columns. The analysis of the retained element or species is carried out later in the laboratory. These procedures would solve the problems of preserving the integrity of the species during sample storage, as expressed earlier on in this section. Already, several methods of this type exist for aluminium, one being the oxine/MIBK extraction method which has been field tested [31].

c) Data on the main aluminium ligands present in any reference water. It is clear that such data, together with their concentrations, should be provided. This is specially important with regards to F⁻, as we have shown earlier.

All of the problems outlined above indicate that the production of a "synthetic" reference water, with well defined total aluminium and Al_{fm} content, could prove most useful for aluminium toxicity control in natural waters before a certified natural water sample of well known and stable aluminium species content becomes available.

In conclusion, a lot of work remains to be done before aluminium speciation analysis in waters becomes a validated and recognised test, able to meet today's requirements, let alone those of tomorrow.

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10.

Selenium speciation analyses in water and sediment matrices

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From the time selenium was discovered in 1817 by Berzelius and Gahn [1] until the recognition in the mid 1950s of its toxicity and, subsequently, its essentiality for animal well-being, a large number of its properties have been elucidated. This element ranks seventieth in abundance among the elements and constitutes approximately $10^{-5}\%$ of the earth's crust. Selenium is located in the VIa Group of the Periodic Table between sulphur and tellurium, and its chemical and physical properties are between these elements, *i.e.* intermediate between nonmetals and the metals, although similarity in behaviour of selenium and sulphur is more pronounced than that of selenium and tellurium. Its atomic number is 34 and its mass of 78.96 results from the six natural isotopes which exist under normal conditions ^{74}Se (0.87%), ^{76}Se (9.02%), ^{77}Se (7.58%), ^{78}Se (23.52%), ^{80}Se (49.82%) and ^{82}Se (9.19%). Other artificial short-lived man-made isotopes such as ^{75}Se , ^{77}Se are utilized in neutron activation and radiology [2]. Selenium has a melting point of 217 °C and a boiling point of 685 °C. Like sulphur it can exist in three allotropic forms: a gray or "metallic", thermodynamically stable, hexagonal form; a red monoclinic form; and a vitreous amorphous form. Selenium is mainly found in metal sulphide deposits, mostly of the metals Cu, Zn, Ag, Hg, and Pb and is obtained industrially as a by-product of the processing of these elements. It is mainly used in the electrical and electronic (23%), rubber, plastics and lubricants (13%), ceramics and glass (27%) and other chemical industries [3], Table 1. Selenium pollution is caused by these industries and by fossil fuel combustion which releases the element into the atmosphere.

Analytical methodologies and applications for the determination of total selenium at trace level have been developed and used extensively in the last thirty years. The reason for determining selenium are multiple: a) the long established selenium poisoning of domestic animals foraging on seleniferous plants, b) disorders in humans and animals resulting from selenium deficiencies and excesses, c) the nutritional essentiality of the element, d) the protective effect of selenium against the toxicity of the metals such as Hg

and As, e) the metabolic interaction between selenium and vitamin E and other antioxidants and f) the reported carcinogenicity, anticarcinogenicity, antimutagenicity and anticlastogenicity and its effect on dietary intake of selenium [4] (Fig.1).

Table 1: Industrial uses of different Se compounds (adapted from ref. [3])

Compound	Uses
Selenium	Solar batteries, photoelectric cells, xerography, stainless steel
Na_2SeO_4	Insecticide, veterinary agent, glass manufacture
Na_2SeO_3	Veterinary agent, glass manufacture, soil additive
Selenium diethyldithio-carbamate	Fungicide
SeS_2	Veterinary agent, shampoos, semiconductors
SeS	Veterinary agent, fungus infection, enczemes
SeF_6	Gaseous electric insulator
SeOCl_2	Solvent
CuSeO_4	Copper alloys
WSe_2	Lubricants
Al_2Se_3 , Bi_2Se_3 , CuSe , InSe	Semiconductors
$(\text{NH}_4)\text{SeO}_3$, As_2Se	Manufacture of red glass
CdSe	Photoconductors, semiconductors, photoelectric cells, solar batteries

It is now well known that it is the chemical form of elements rather than their concentration which determines their role in the living organism. This is particularly so for elements such as, Se, Hg, *etc.*, which exist in many different forms and oxidation states.

Natural waters, sediments and air are the most commonly studied environmental media. Robberecht and Van Grieken [5] have extensively reviewed the literature on total selenium in natural waters of river (content 0.016-20 ng.l⁻¹), lake (< 0.1-1.85 ng.l⁻¹), open ocean (0.025-0.2 ng.l⁻¹) and coastal or harbour environments, but an exhaustive review of selenium species in environmental samples has not yet been published.

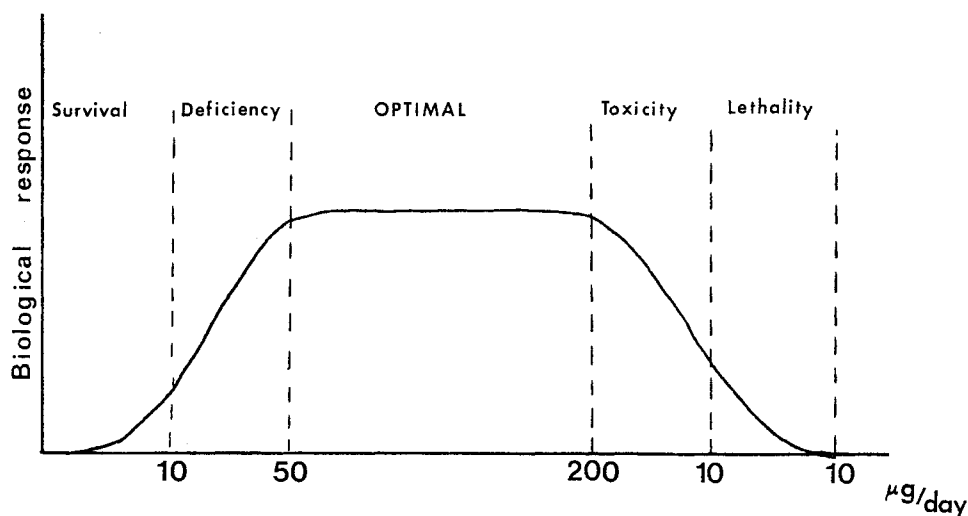


Figure 1: Concentration range of essentiality or toxicity of Se (daily intake)

10.1 Selenium species in the environment

Selenium is a trace element which can exist in four oxidation states in the environment: selenite (Se(IV)^+), selenate (Se(VI)^+), elemental selenium (Se(0)) and selenide (Se(II)^-) [6,7]. These oxidation states can be found in particles and sediments; therefore, selenite and selenate can either be adsorbed onto particles or exist as metal selenites or selenates. Selenides can be found as inorganic selenides, usually FeSe (achavalite) or FeSe_2 (ferroselite), or as organic forms such as selenoaminoacids. These particles act as an "elemental reservoir" and may release the different chemical forms of the element into natural water (river, estuary or sea) through an adsorption/desorption process that has been extensively studied. The principal factors that govern these processes are: the way of binding of the element with the particulate matter, oxidation state, water-sediment interface, redox potential and pH [5,8].

The dissolved selenium species in natural water (mainly seawater) have been studied [9,10] and there is some evidence that colloidal inorganic and organic forms exist as well.

The pH-E diagrams for selenium in aqueous systems reveal that over a wide range of potential and pH the quadrivalent form of selenium is the most stable (*i.e.* pH 8.1 and pE 12.5). In oxic marine water selenate should be the exclusive oxidation state. On the contrary, under anoxic reducing conditions, selenide should become the stable dissolved form, although the presence of Se(0) or inorganic Se(II)^- have not been established [11-16]. However, in addition to thermodynamic factors, the existence of kinetic effects and the biologically mediated production of unstable species may change the picture.

The contribution of colloidal inorganic selenium to total dissolved inorganic selenium decreases with increasing salinity. A study by Takayanagui *et al.* [14] in the Southern Chesapeake Bay and adjacent waters showed that colloidal inorganic selenium constitutes 77, 40 and 0% of the total dissolved inorganic selenium in river water, estuarine water

and coastal seawater, respectively and that most of the colloidal inorganic selenium is found in material of nominal molecular weight between 1000 and 5000. Organic selenium was also found in the three types of water and colloidal organic selenium constitutes 70, 64 and 35 % of total dissolved organic selenium in river water, estuarine water and coastal seawater, respectively. The contribution to total dissolved organic selenium again decreases with increasing salinity [17]. The relationship between selenite, dissolved inorganic selenium and dissolved total selenium in the upper St Lawrence Estuary [18] showed that selenite was the predominant species in the river environment, while selenate, selenite and organic selenium shared significant fractions of the total selenium in the oceanic area. An examination of the total dissolved selenium distribution with depth reveals (as well as other trace elements) depletion in the surface water and enrichment with depth. This is typically explained by a process of incorporation of dissolved elements into biogenic particulates in the surface water, vertical transport and regeneration of the dissolved state in deeper waters. Takayanagi and Wong [19] showed that the distribution of Se(IV), Se(VI) and dissolved organic selenium species can be explained by the biological uptake of Se in the surface waters and the multi-step regeneration of Se from biogenic particles at greater depths.

Although the specific biochemical function of selenium in marine organisms is not well understood, selenoaminoacids can be found as a result of biogeochemical processes by microorganisms [20]. Wrench [21] has observed that some marine phytoplankton are capable of incorporating selenium to form selenoaminoacids and Cutter [12] detected selenium in dissolved amino acids collected from the waters of the Saanich Inlet.

The biomethylation of selenium may also produce compounds such as dimethylselenide, dimethyldiselenide, dimethylselenone, *etc.* [22], and different mechanisms of production of methylselenide and dimethyldiselenide in plants have been proposed [23, 24]. Methylation is associated with the growth of microorganisms and is affected by temperature [25], oxygen [26], sunlight and pH [24] with optimum biomethylation occurring at 40 °C and pH 7.8.

Dimethylselenide and dimethyldiselenide are volatile compounds that are released into the air at rates depending on the location [27,22,28]. These compounds and volatile selenium emitted by man (about 40% of all selenium released) [29,30] are the most significant sources of atmospheric selenium. These exchanges and conversion processes among the species are illustrated in Fig.2.

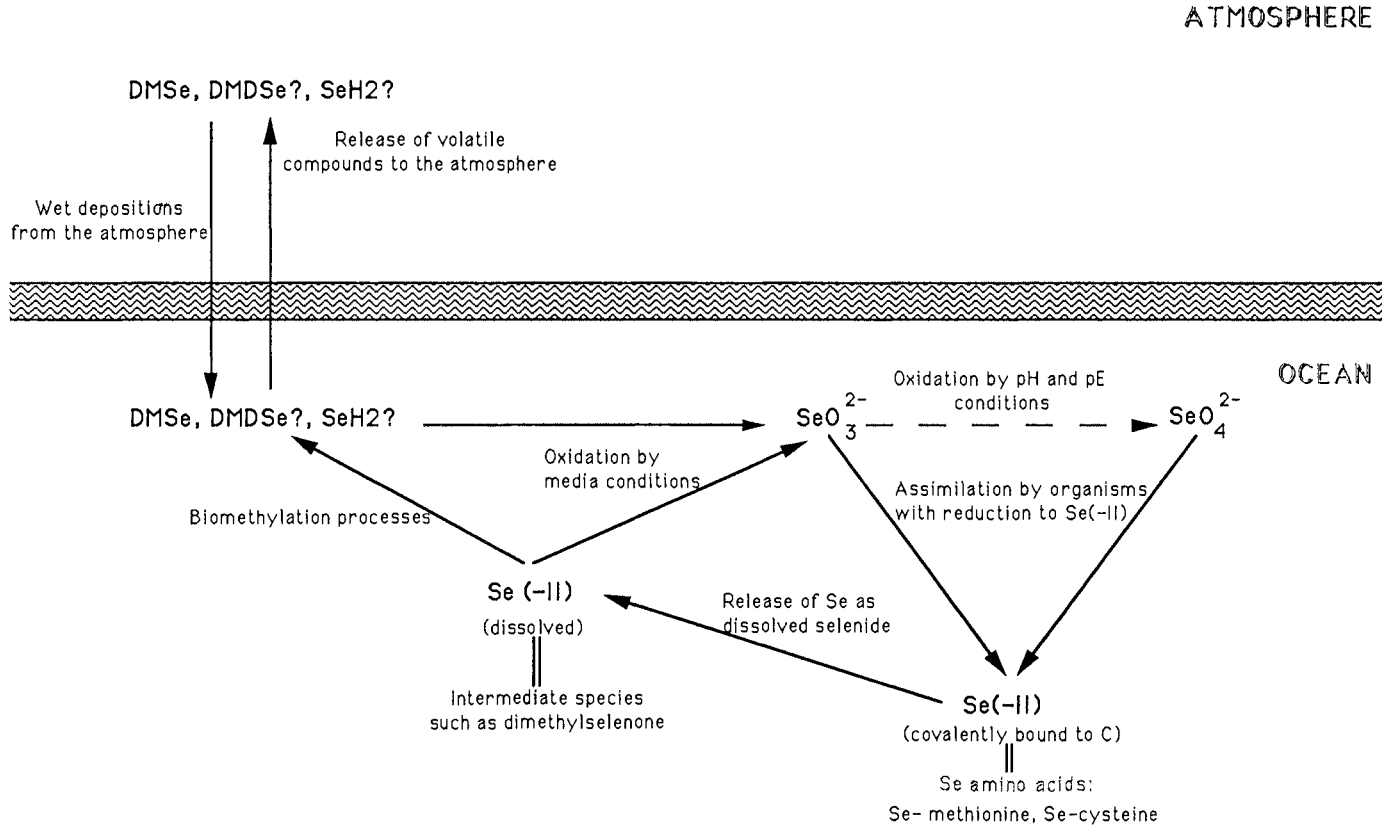


Figure 2:

Schematic biogeochemical cycle of selenium in the ocean-atmosphere exchanges (adapted from [107])

10.2 Analytical methodology - General remarks

The development of sufficiently sensitive analytical methods for selenium species is a challenge. The problems to be overcome arise from: (i) the low concentration of the species to be determined and (ii) the lack of knowledge of sampling and storage. The factors which affect the sorption or loss of selenium or interconversion of one species into another during sampling, sample storage and sample analysis may be classified into four categories according to Massee *et al.* [39].

The analyte itself, especially because of its chemical form and concentration, demands simple procedures for high sensitivity determinations. Characteristics of the solution, such as the presence of acids (pH), dissolved material (e.g. salinity, hardness), complexing agents, dissolved gases (especially oxygen, which may influence oxidation state), suspended matter (competitor in the sorption processes) and microorganisms (e.g. algae). In addition, properties of the container, such as chemical composition, surface roughness, surface cleanliness and surface area/volume ratio. The history of the container such as age, cleaning method, exposure to heat, *etc.* is important because it may directly influence the type and number of active sites for sorption. Finally, external factors, such as temperature, contact time, access of light and occurrence of agitation have an influence.

10.3 Method validation

The major factor in speciation analysis is quality assurance, *i.e.* method validation. The analytical methods developed for Se speciation can be tested as follows:

- (i) using certified reference materials: unfortunately, this alternative is not always achievable due to the lack of certified reference materials of selenium species.
- (ii) natural samples spiked with the species to be determined, but the analyst's knowledge of the amount added may unintentionally influence the result.
- (iii) using alternative methods: this approach is not common because of the lack of widely different methods within the same laboratory.
- (iv) interlaboratory comparisons: the same sample is analyzed by laboratories using different methods. This approach has not been considered so far for Se speciation and is currently investigated by the Measurements and Testing Programme (BCR).
- (v) using blanks: although this gives a few problems in trace analysis, in ultratrace and speciation analysis the blank signal is often comparable with that of the sample. Thus any uncertainty in the blank is directly reflected in the analytical result. We distinguish three types of blanks: a) known blanks, for which correction is possible with complete confidence and often without significant loss of precision; b) estimated blanks, which depend on supplementary information on the particular sample analyzed and for which correction is more uncertain, although a maximum error can usually be given; and c) unknown blanks, for which no correction can be applied even when potential sources of blank errors are known, the only solution being to reduce the contribution of individual blanks to an insignificant level.

When species must be preconcentrated to get the desired sensitivity, further validation problems arise because the simultaneous preconcentration of the desired species with the preconcentration of the undesired species may influence the results. This is a common failure in publications proposing new methods.

This section dealing with the validation of methods for determining inorganic Se, Se(IV) and Se(VI) and organic species, will review the current Se speciation methods, discuss their advantages, limitations and feasibility and examine the interferences arising when validation is possible. An overview of species preservation and the proposed preservation methods is included.

10.4 Stability of selenium species

Although the US Environmental Protection agency [31] has recommended nitric acid at $\text{pH} < 2$ for the preservation of Se in natural waters, the losses observed in polyethylene containers under these conditions and the fact that nitrate interferes with the hydride generation method for selenium led to controversial results in the first studies on Se species stability and underlined the need for investigating means of preservation of species in relation to the water type, concentration, temperature, pH and the properties of the containers.

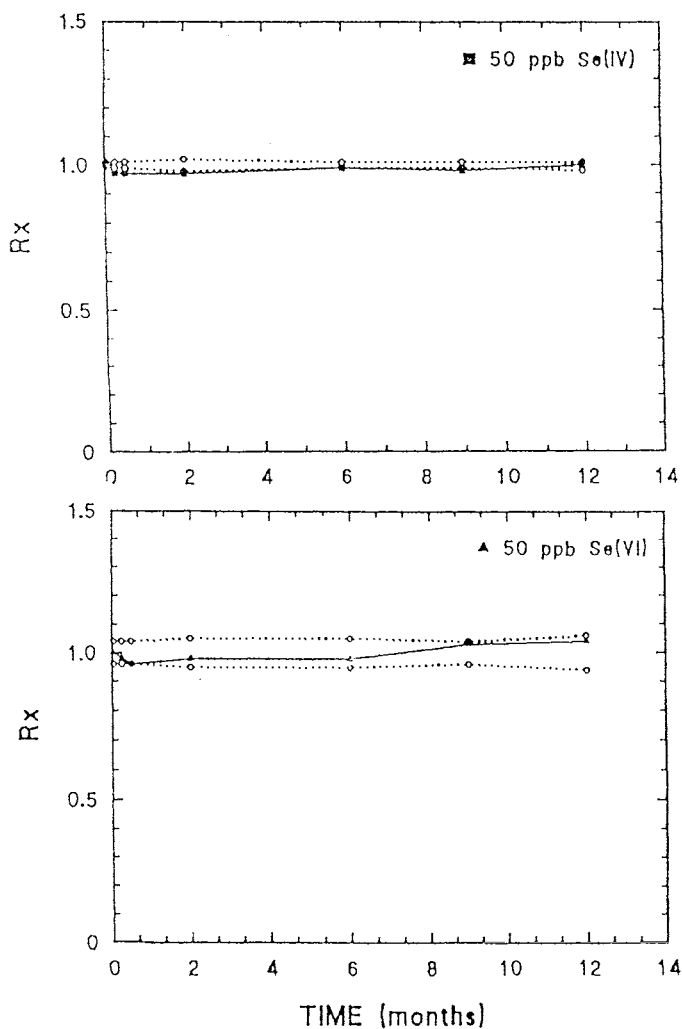
Shendrikar and West [32] have reported 4% losses of Se(IV) in 15 days from distilled water at $\text{pH}=7$ in flint glass and 8% in polyethylene container. Cheam and Agemian [33] reported that the stability of moities of 1 and $10 \mu\text{g.l}^{-1}$ of Se(IV) and Se(VI) species at pH 1.5 in sulphuric acid media in 500 ml pyrex or polyethylene bottles was satisfactory over 125 days. For $1 \mu\text{g.l}^{-1}$ Se(IV) at pH 5.4 losses occurred in both pyrex and polyethylene bottles, whereas complete recovery was obtained in 25 gallon barrels for distilled and natural (harbour) water. This indicates that the size of the container, or rather its surface area per unit volume, directly influences the Se(IV) stability. The results obtained at $10 \mu\text{g.l}^{-1}$ were the same than that obtained at $1 \mu\text{g.l}^{-1}$ but the tenfold increase in Se(IV) concentration decreases losses at higher pH. Dissolved salts is a better preservative for Se(IV) than distilled water because surface interaction is lower. Schutz *et al.* [34] observed no loss of selenium from natural seawater and Measures *et al.* [35] reported constant inorganic Se(IV) and Se(VI) content in seawater samples acidified to pH 2 with hydrochloric acid and stored in glass or polyethylene containers for 4.5 months. An interlaboratory quality control study [36] using sample acidification of 0.02 % v/v sulphuric acid and storage at room temperature in polyethylene bottles found excellent recoveries over the 0-1000 $\mu\text{g.l}^{-1}$ selenium concentration range and confirmed the effectiveness of the proposed preservation method. High acid concentration, such as 32% HCl, enables the storage of natural water in polyethylene containers for several weeks without significant losses of selenite and selenate [37]. May *et al.* [38] found no apparent change in Se(IV) concentration after 50 days storage of 2 mg.l^{-1} solution in polyethylene or borosilicate glass containers in 15% hydrochloric or 5% sulphuric or mixed HCl-H₂SO₄ acid. Massee *et al.* [30] showed that Se(IV) stability at the $\mu\text{g.l}^{-1}$ level for 28 days was independent of pH (pH 8.5 with nitric acid or sodium hydroxide), container type (borosilicate glass, high-pressure polyethylene or polytetrafluoroethylene), surface area to sample volume ratio and sample matrix (distilled or artificial seawater). The observed stability of Se species may be because Se(IV) and Se(VI) form oxyacids which are partly dissociated, leading to negatively charged ions.

Cutter [40] studied the Se speciation in biogenic particles and sediments and concluded that the stability of selenite and selenate during the storage of spiked samples (30 ml linear polyethylene bottles, 10 ml HCl/water at pH 1.6, $1.2 \mu\text{g.l}^{-1}$ of Se as selenite or selenate, refrigerated storage), prevented microbial growth and speciation changes for 3 weeks. Dunju *et al.* [41] showed that quartz containers are better than polyethylene (PE)

and glass containers for preserving both species in acidified water samples. No concentration changes were observed for Se(IV) at $0.06\text{--}0.13\mu\text{g.l}^{-1}$ and Se(VI) at $0.03\text{--}0.13\mu\text{g.l}^{-1}$ in water samples at pH 1.1 containing up to 3.2 mg.l^{-1} fulvic acids in polyethylene containers for 30 days.

May *et al.* [38] studied the matrix-dependent instability of selenium (IV) stored in PTFE containers in different acidic matrices (HCl , $\text{HCl}+\text{H}_2\text{SO}_4$) and found that the combination of Se(IV), PTFE and H_2SO_4 acid should be avoided when preparing samples for speciation purposes. These results clearly differ from Cobo *et al.* [42], which showed a similar phenomenon but occurring at pH 6 (Fig.3).

Fig.3: Stability of the inorganic Se species in solutions stored in polyethylene containers at pH 6, 20°C in the dark (adapted from [42])



In this study on inorganic selenium species stability at 10 and 50 $\mu\text{g.l}^{-1}$ at $-20\text{ }^{\circ}\text{C}$, $20\text{ }^{\circ}\text{C}$ and $40\text{ }^{\circ}\text{C}$, at pH 2 and 6 in the absence and presence of chloride and in different containers, the authors concluded: the stability of 10 and 50 $\mu\text{g.l}^{-1}$ moities at pH 6 in polyethylene containers seems to be higher than at pH 2 (no losses of Se(IV) were detected over a two month period whereas losses of Se(IV) started after 1 month of storage and were complete after a year). Considering that at pH 2, Se(IV) species were stable for one year and algae cannot grow, the Se(IV) losses might be attributed to adsorption onto the container walls. The presence of chloride decreases the risk of Se(IV) losses. A recent study [43] has shown that addition of chloride to a final concentration of 5000 mg.l^{-1} clearly stabilized both Se species at 10 and 50 $\mu\text{g.l}^{-1}$ at pH 6 and pH 2 (obtained with HCl) stored in polyethylene containers for the 4 months tested.

Significant Se(IV) losses were observed at pH 6 in PTFE containers (higher at 10 $\mu\text{g.l}^{-1}$ than at 50 $\mu\text{g.l}^{-1}$). The increase of Se(VI) concentration observed suggested some transformation of Se(IV) into Se(VI). In contrast, Se(IV) species are stable at pH 2. It was concluded that both species are clearly stabilized at pH 2 in PTFE container.

Both species were stable at $-20\text{ }^{\circ}\text{C}$ over 12 months and losses of Se(IV) and Se(VI) were lower at $40\text{ }^{\circ}\text{C}$ than at $20\text{ }^{\circ}\text{C}$. In both cases losses depended on the pH and the type of container. These results are summarized in Table 2.

Table 2: Stability of Se species under different storage conditions

Species	Conc.	pH	Matrix	T ($^{\circ}\text{C}$)	Storage time	Container	Ref.
Se(IV)		7	Distilled water		15 days	glass or polyethylene	[32]
Se(IV) Se(VI)	1-10 $\mu\text{g.l}^{-1}$	1.5	H_2SO_4		125 days	pyrex or polyethylene	[33]
Se(IV) Se(VI)		2	seawater (HCl)		135 days	glass and polyethylene	[35]
total Se	0-1000 $\mu\text{g.l}^{-1}$	1	H_2SO_4	room temp.	several weeks	polyethylene	[36]
Se(IV)	2 mg.l^{-1}	1.5	HCl or H_2SO_4		50 days	pyrex glass or polyethylene	[38]
Se(IV) Se(VI)	1-2 $\mu\text{g.l}^{-1}$	1.6	sediment	$4\text{ }^{\circ}\text{C}$	21 days	polyethylene	[40]
Se(IV) Se(VI)	0.06-1.5 $\mu\text{g.l}^{-1}$	1.1	water (3 mg.l^{-1} of fulvic acids)	room temp.	30 days	polyethylene	[41]
Se(IV) Se(VI)	10-50 $\mu\text{g.l}^{-1}$	2 and 6	NaCl 100 mg.l^{-1}	$-20\text{ }^{\circ}\text{C}$	365 days	polyethylene	[42]

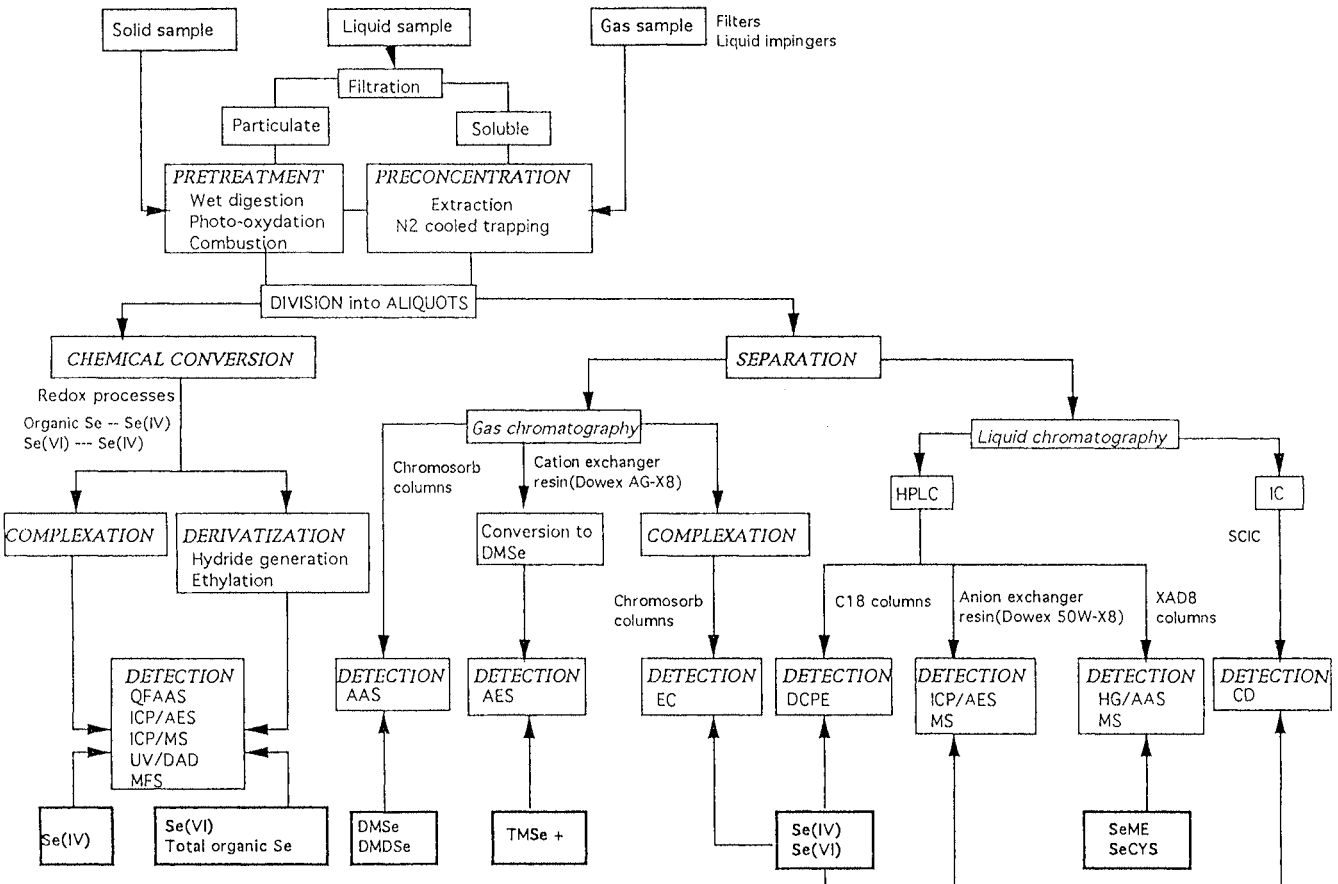


Figure 4: Scheme of analytical procedures used for Se-speciation in a wide variety of matrices (adapted from [108])

10.5 Analytical methods for selenium speciation

10.5.1 Fluorimetric methods

Fluorimetric methods for selenium speciation in natural waters have been extensively used for the determination of selenium in polluted [44] and unpolluted waters [9,45,46] and the chemistry of spectrofluorimetric determination has been widely studied [47,48]. Se(IV) is complexed with 2,3 diaminonaphthalene to form 4,5 benzopiazselenol. The complex is extracted into cyclohexane and the fluorescence intensity is measured at an excitation wavelength of 380 nm and an emission wavelength of 520 nm. Only Se(IV) can be determined by this method, so speciation is limited by the ability to transform each species to Se(IV).

Fluorimetric speciation has been extensively used by Takayanagi *et al.* [14,17-19,49] and others [50] in research on the geochemistry of Se species in marine and estuarine environments. The forms differentiated were inorganic selenium, such as Se(IV), Se(VI) and Se(O), organic selenium, and inorganic and organic colloidal selenium. Figure 5 summarizes the speciation studies.

In this technique, each sample is filtered through a precombusted Gelman A/E glass fibre (G/F) filter immediately after sample collection. The subsample 1 of this filtrate are then filtered through an AMICON DIAFLO UM2 membrane of approximately 1.2 nm nominal pore size. Since the particle size of colloidal material typically ranges from 1 nm to 1 μm , colloidal particles pass through the first filter and are retained in the second. Colloidal species which pass through the second filter are considered to be dissolved material. Aliquots of subsamples 2 are oxidized by UV irradiation from a 1200 watt mercury lamp for at least 5 h in the presence of hydrogen peroxide. More than 92% of dissolved organic carbon is destroyed by this procedure and organic selenium is transformed into inorganic species; HCl reduction then converts Se to Se(IV). This aliquot contains all Se species except colloidal forms. The inorganic speciation of Se(IV) and total inorganic Se in aliquots of subsample 2 not exposed to UV radiation can be performed, although usually a preconcentration step is required because the sensitivity of fluorimetric methods is far from sufficient to measure the usual concentrations in natural waters. Se(IV) can be directly preconcentrated from 500 ml or 1 l of water by extracting its complex with ammonium 1-pyrrolidine dithiocarbamate (APDC) into chloroform and back-extracting the selenium into nitric acid [14]. APDC reacts with Se(IV) but not with Se(VI) at the selected pH of 4.2 [50]. The fluorimetric method for Se(IV) is then applied.

In a duplicate aliquot the total inorganic selenium is preconcentrated using a tellurium solution. The inorganic selenium species are reduced to the elemental state and coprecipitated with tellurium in the presence of hydrazine sulphate. The precipitate is dissolved in a mixture of nitric and perchloric acids and, after reduction of all species to Se(IV) with HCl, the fluorimetric method is applied. By this procedure, the sum of Se(O) has not been detected in oxic natural waters and Se(VI) species can be determined as the difference between total inorganic selenium and Se(IV). The detection limit after preconcentration of the sample, calculated as the concentration which produces a signal to noise ratio of 2, was 20 pmol.l^{-1} . Precision, measured as the relative standard deviation, was about 2% at concentrations above 274 pmol.l^{-1} and 8% at 131 pmol.l^{-1} . Recoveries averaged about 92% and were computed for samples processed as described by comparing calibration slopes or concentrations, with solutions being prepared with the same final concentrations without preconcentration.

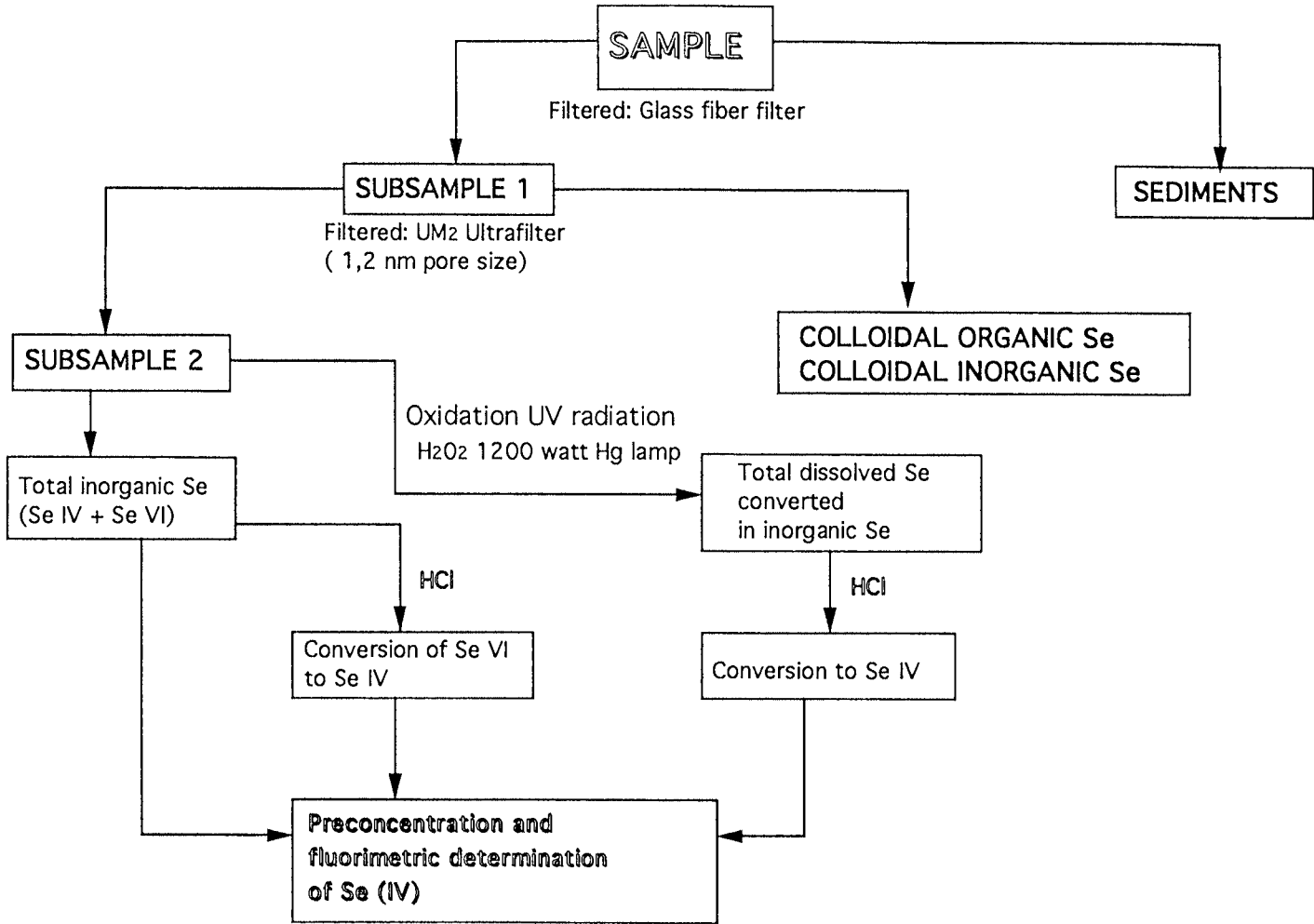


Figure 5: Speciation scheme for selenium speciation by fluorimetric determination.

Some errors may occur in the determination of Se(IV) and Se(VI) by fluorimetric methods after preconcentration: in river water, high concentrations of dissolved organic carbon are common and fine precipitates are formed in the preconcentration step. Therefore, standard addition procedures are recommended for each sample. In addition, when Se(IV) is preconcentrated in chloroform solvent, organic matter and organic selenium species, such as selenium associated with fatty acids, hydrocarbons, lipids and some humic substances, may be also extracted, so the concentration of selenium measured includes Se(IV) and some of the organic selenium forms in samples. Another possible source of error identified by Takayanagi *et al.* [17] was due to the use of hydrogen peroxide or bromine to convert inorganic selenium species to the +4 state. An unknown amount of organic selenium may also be converted to the inorganic form. Thus, Se(VI), calculated as the difference between total selenium and Se(IV) may include organic selenium as well as Se(VI).

The total dissolved non-colloidal organic forms of Se are calculated as the difference between the total selenium in the UV irradiated aliquot and the total inorganic selenium in the non-irradiated aliquot. These organic forms of selenium may include methylated species; although the predominant forms are more likely Se-amino acids or Se-peptides. The occurrence of monomethylselenide and dimethyldiselenide is not frequent in natural water samples.

Total colloidal organic selenium is calculated as the difference between the organic selenium in the filtrate from the glass-fibre filter and that from the UM2 ultrafilter. A study on coastal seawater showed that colloidal organic selenium has nominal molecular weight (NMW) of about 1000.

Total inorganic colloidal selenium is calculated as the difference between the total inorganic selenium in the solution after ultrafilter separation and that in the solution collected from the fibre filter.

10.5.2 Electrothermal atomic absorption spectrometry

Electrothermal atomization adsorption spectrometry (ETAAS) has often been used for the determination of total selenium owing to its high sensitivity. It may be applied to the determination of Se(IV) and Se(VI), providing that chemical reactions are carried out prior to the detection such as *e.g.* complex formation followed by solvent extraction, or specific retention of certain species on solid supports which are normally required to achieve species separation.

The determination of inorganic selenium involves a previous chemical reaction which is only useful for one oxidation state, and the other must be obtained as the difference between total selenium and the determined species. The error for the species determined by difference is always much higher than for the species determined directly.

The analytical methods proposed by different authors are mainly based on the extraction of one inorganic selenium species and differ mainly in the organic reagent used to complex Se(IV) or in the organic solvent used as extracting and/or on the way that inorganic selenium species are converted to Se(IV).

Ammonium pirrolidindithiocarbamate (APDC), diethyldithiocarbamate (DDTC) and dithizone are the reagents most commonly used for the complexation of Se(IV) [51-55]. Chloroform (Cl_3CH), carbontetrachloride (Cl_4C) and methylisobutylketone (MIBK) are the usual extraction solvents. The joining effects of APDC- Cl_3CH - Cl_4C [53] allows to extract selectively Se(IV) without extracting Se(VI) in an acidity range of 5 mol.l⁻¹ HCl to pH 7. The mixture of APDC-MIBK selectively extracts Se(IV) [54] from an aqueous

solution at pH 3.5 to 5 and the DDTC-Cl₄C mixture extracts this species from solutions containing citrate-EDTA [55]. The 4-chloro-1,2 diaminobenzene is also a reagent used for the Se(IV) extraction [56].

The determination of total selenium is performed prior to the reduction of Se(VI) to Se(IV). Although some reagents have been proposed for this purpose, such as 4% TiCl₃ [53], hydrogen peroxide [56], the addition of HCl is the most reliable way to perform the reduction step, Se(VI) is quantitatively reduced to Se(IV) by heating on a boiling water bath for 15-20 minutes in 4-6 mol.l⁻¹ HCl [54,56]. Total selenium converted to Se(IV) is similarly determined and after Se(VI) concentration is obtained by difference between total Se and Se(IV). Sometimes, preconcentration of total selenium is performed by a simple evaporation step in the presence of HNO₃ to minimize the risk of selenium losses. The presence of this acid may result in the hydrolysis of any organoselenium compounds and the oxidation of Se(II) and elemental selenium to Se(IV) or Se(VI), and thus may introduce significant errors [56].

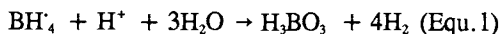
The addition of Cu(II) or Ni(II) as matrix modifier to decrease selenium volatility in the graphite furnace is very useful. The mechanism of these elements is not well understood but probably the formation of compounds such as CuSe, NiSe, reduces the selenium volatility. Detection limits of Se species are around 1 µg.l⁻¹ or lower [54]. The precision is around 5%. These methods were applied to the speciation of inorganic selenium in drinking, river, sea and waste waters.

Another procedure for the separation of Se(IV) and Se(VI) species used an anion exchange resin [57]. Se(VI) was retained on the column (Ag-1X8 anion exchange resin) in the presence of 0.05 mol.l⁻¹ HCl while Se(IV) was eluted. Se(VI) was subsequently eluted from the anion-exchange column with 30 ml of 0.3 mol.l⁻¹ HCl. Then the different fractions were analyzed by ETAAS. This method was applied to air samples: selenium was adsorbed on a column of gold-coated quartz beads, then Se(IV) and Se(VI) were leached with warm distilled water and elemental selenium was removed from the column with 3 mol.l⁻¹ HNO₃; the detection limit was 0.03 ng.m⁻³.

10.5.3 *Hydride generation / atomic detectors*

Hydride generation (HG), coupled to AAS and inductively coupled plasma (ICP) as the main atomic detectors, is the technique most commonly used for inorganic Se determination. It does not require extensive sample pretreatment and its sensitivity, in the ng.l⁻¹ range, is suitable for environmental concentrations.

The reaction of Se(IV) and the reductant, sodium borohydride (NaBH₄), in acid medium yields to volatile SeH₂. The generation of hydrogen selenide proceeds according to the following reactions:



Since this reaction is specific for Se(IV), hydride techniques require the prior conversion of Se species to Se(IV).

The hydride can be atomised in:

- an air-acetylene flame;
- a heated quartz cell with a mixed hydrogen-oxygen flow to achieve complete decomposition of SeH_2 bonds [58]. Some excess of hydrogen is required to prevent this drop in the absorbance;
- a graphite furnace that gives good Se atomisation and eliminates matrix interferences.

Hydrogen is generated at the same time as SeH_2 and acts as an additional carrier. Over vigorous H_2 formation causes irreproducible signals, probably due to an excess of SeH_2 formation. The main factor governing H_2 generation is the NaBH_4 concentration [59]. The acid, or more commonly, hydrochloric acid.

NaBH_4 must be stabilized, usually with sodium hydroxide, to prevent hydrolysis. Solutions stabilised with 2% w/v NaOH can be stored for at least 8 weeks without losses of reducing activity [59]. The optimal sodium borohydride concentration is 3.5-5% w/v in batch systems, and 0.2-0.4% w/v in continuous systems [60]. The system is generally purged with an inert gas (He or Ar), which is of great importance in the determination of Se. The Se absorbance is measured in the far ultraviolet region of the spectrum where the absorbance by air is considerable.

Total inorganic selenium is determined, as in the above mentioned techniques, by prior quantitative reduction of Se(VI) to Se(IV). Determination of Se(VI) is obtained as the difference between total selenium and Se(IV). Several reductant mixtures, such as stannane chloride - potassium iodide, ammonium chloride - hydrochloric acid and sulphuric-hydrochloric acid gave interferences when used with NaBH_4 for hydride generation. Nowadays, quantitative reduction is achieved by simply boiling a solution of Se(VI), acidified with 4-6 mol.l⁻¹ of HCl, for 10-20 min [61].

Hydride generation of selenium can be carried out by batch process, FIA or by a sampling continuous flow system. The advantages of FIA (full automation, quick analysis, minimum analyte and reagent consumption, exactly defined sample volume) are added to the advantage of hydride generation (separation of the analyte from its matrix), Fig.6.

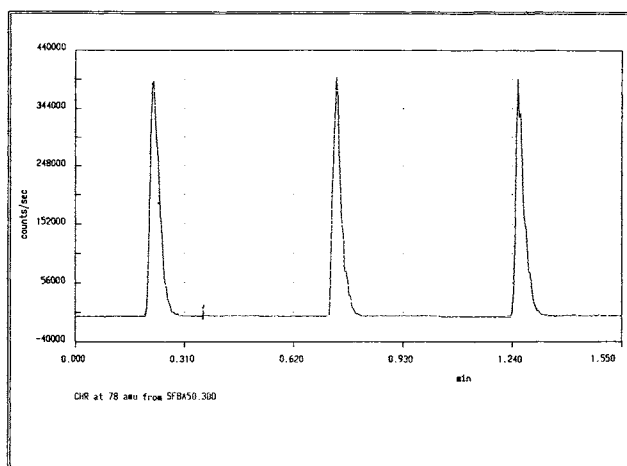


Figure 6: Signal obtained by HG/FIA/ICP/MS for a concentration of 1 ng.l⁻¹ of Se. Repeatability of three replicates [111]

Recently an on-line reduction using FIA and continuous sampling flow has been proposed [62]. The method has been applied to seawater analysis and requires the addition of 12 mol.l⁻¹ HCl for the reduction of Se(VI) to Se(IV) and for the hydride generation. The reaction loop is heated at 140 °C in a graphite bath. The reduction is complete and Se(VI) is determined as the difference between total selenium and Se(IV) (determined without heating). The detection limits are 0.7 µg.l⁻¹ for Se(IV) and Se(VI) in the FIA system and 1.5 µg.l⁻¹ when the continuous sampling flow system is used. Interferences from metal are decreased by using concentrated HCl.

Cold trapping (CT) was used for SeH₂ preconcentration in environmental samples and for speciation of other volatile species by connecting the trap to a chromatographic column [63]. As explained above, selenium hydride is generated in a reaction vessel and carried by a stream of the carrier gas to the condenser, which is immersed in liquid nitrogen. Drying agents are necessary to remove water vapour, before collecting the hydride, and avoid ice clogging in the cold trap. Reagents such as NaOH, CaSO₄, CaCl₂, Mg (ClO₄)₂ and H₂SO₄ have been used. Risks of reagent contamination have been observed and have to be considered carefully [64]. Normally a trap consisting of a tube immersed in a dry ice-isopropanol bath is satisfactory [61]. During the reaction, the condenser is kept immersed in the liquid nitrogen to trap the volatile SeH₂ species. When the reaction is complete the cold trap is removed and the hydride rapidly volatilizes and is driven to the AAS or ICP by the carrier gas [65].

Difficulties may arise in the cold trap technique due to the limited stability of the hydride [64], which is so reactive that most systems are unable to fully elute it from the chromatographic column. Other volatile substances present in the sample or formed during hydride generation are also collected in the trap and may interfere with the determination of the analyte [63]. Also, these byproducts (CO₂, volatile boranes, diborane, HCl, *etc.*) may shorten the column life time.

A new multielement photo-ionization approach can be used to replace AAS and ICP detection. A continuous flow hydride generator is coupled to a cryogenic trap to improve sensitivity. After sample collection in the liquid nitrogen trap, the hydrides formed are separated by gas chromatography (GC) and determined by photo-ionization detector. At least 4 elements have been analysed simultaneously by this technique: Se, As, Sn, Sb [64]. The detection limit of 1-2 ng.l⁻¹ is one of the best reported so far.

ICP-MS has a great potential for Se analysis but it has not been fully explored for selenium speciation. Sample introduction by nebulization is limited owing to the low sensitivity obtained. With classical nebulization systems, not only molecular ions of the same mass/charge ratio as the analyte in the sample matrix, but also reagents, water and carrier gas may interfere. For example, HCl present in the samples may be carried by the aerosol and reach the plasma, forming Ar⁴⁰Cl³⁷⁺ which increases the background intensity at m/z 77. Hydride generation as a sample introduction method may overcome this problem as the precursors do not reach the plasma torch [60]. Se has been determined by isotope dilution using ⁷⁶Se as the reference isotope and enriched ⁷⁶Se or ⁸²Se as the tracers. The detection limit is 1.3 ng.g⁻¹ for plant materials. The validation was performed using biological Reference Materials (IAEA, NIST).

10.5.3.1 Interferences

Interferences affecting Se determination by hydride generation may appear at different times: during hydride generation from the sample matrix, (due to the presence of transition metals or other hydride-forming elements); after hydride formation (due to adsorption or complexation phenomena); and in the atomisation step (due to the competition for consumption of H radicals by other species).

Co(II), Cu(II), Sn(II), Sb(III), and Ni(II) ions cause some signal depression when they are present before the reaction, although their effects are moderate and only copper has a pronounced influence on the selenium signal [66,67]. The reason of this signal loss is the formation of stable complexes in a gas-liquid reaction. The interfering elements are not likely to compete with Se in the reduction, because NaBH_4 is always present in excess in comparison to the interfering ions [67]. Agterdenbos *et al.* [68] suggested that transition metals decompose the NaBH_4 before the reaction is complete. They also proposed the addition of iodide to the sample, since it catalyzes SeH_2 formation even in the presence of interfering metal ions.

Cutter [69] found that nitrite interferes seriously in Se determination at environmental concentrations, and explained that this interference was due to the reaction of nitrite and the generated hydride. Also, nitrate can interfere indirectly in the determination of selenate, because some nitrate is reduced to nitrite during the reduction of Se(VI) to Se(IV). The addition of sulphanilamide completely eliminates these interferences.

Organic substances may also suppress the Se signal, probably due to the formation of an adduct involving SeH_2 and an organic constituent of the sample matrix, such as humic acids. Interferences may also arise through the inhibition of SeH_2 , as a result of strong interaction between Se and the organic compounds formation. Roden and Tallman [70] observed that the interfering constituents can be retained on a XAD-8 column, while Se is not. The interferences in the atomization step of AAS have been extensively studied by Barth and Krivan [71], who showed that interferents can modify the surface of the atomiser and greatly influence the analyte absorption signal. Furthermore, the interferences by other hydride-forming elements in Se detection can be reduced by the addition of oxygen, which gives rise to O and OH radicals thereby increasing the H population in the atomiser and resulting in a better atomisation.

10.5.3.2 Ethylation

An alternative to the hydride method for the derivatization of several metal ions (Hg, Pb, Sn) is ethylation using sodium tetraethylborate ($\text{NaB}(\text{C}_2\text{H}_5)_4$) reductant. This method has been reported by Clark and Craig [72] for the determination of Se. They showed that $\text{NaB}(\text{C}_2\text{H}_5)_4$ can be used to prepare Se compounds for speciation analysis by GC/AAS or GC/MS. The production of a single derivative $(\text{C}_2\text{H}_5)_2\text{Se}$ from the Se(IV) and Se(VI) oxidation states suggests the reduction to Se(II) followed by ethyl carbanion attack to yield the corresponding derivative. Se(VI) is derivatized directly by this method, whereas, in the case of hydride generation, it is not reduced by NaBH_4 and hence necessitates a further reduction step. However, the $(\text{C}_2\text{H}_5)_2\text{Se}$ method does not allow the speciation of two inorganic Se species.

Photo-oxidation of the sample to release the organic material present in seawater resulted in very large increases in available Se(IV) due to the oxidation of dissolved organic selenium.

This method has been used for the routine analysis of both Se(IV) and total Se in seawater and its detection limit is 1 ng.l^{-1} .

10.5.4 Hyphenated techniques

10.5.4.1 Liquid chromatography/ Atomic detectors

Chromatographic methods, especially HPLC (high performance liquid chromatography), coupled with different detection systems have given a great impulse to selenium speciation. The main species separated by HPLC are selenite, selenate, trimethyl-selenium. The atomic detectors most widely used are AAS and ICP.

Mehra and Frankenberg [73] developed a chromatographic method for Se(IV) and Se(VI) speciation by single column ion chromatography (IC). They used a low capacity resin-based anion-exchange column and p-hydroxybenzoic acid (4 mmol.l^{-1}) at pH 8 as eluent and quantified the species with a conductivity detector. The detection limits for a 2 ml of sample injection were 60 and $110 \text{ }\mu\text{g.l}^{-1}$ for Se(VI), respectively. Anions such as Cl^- , NO_3^- , and SO_4^{2-} did not interfere. In contrast to previous SCIC works [74, 75] these anions interfered in the quantification of Se(VI). The method was applied to analyze seleniferous soil samples.

The use of a derivatiser [76] or a specific selenium detector [70,79] increases selectivity and eliminates the effect of compounds which interfere when, for instance, a conductivity detector is used. Furthermore, the increased sensitivity makes it possible to analyze real samples containing low levels of selenium.

Shibata *et al.* [76] used an ion-exchange chromatography for the separation of Se(IV) and Se(VI), spectrofluorimetry for post-column detection and 0.2% (w/v) 2,3-diaminonaphthalene (DAN) as a derivatiser. As DAN reacts only with Se(IV), an on-line prerelution step was proposed to reduce Se(VI) to Se(IV) using a HBr reductant and a reduction loop heated at 100°C . The detection limits were $0.17 \text{ }\mu\text{g.l}^{-1}$ for Se(VI) and $0.5 \text{ }\mu\text{g.l}^{-1}$ for Se(IV) when $200 \text{ }\mu\text{l}$ are injected. The method was applied to tap water.

Chakraborti *et al.* [74] used an anion precolumn (Dionex 30008) to concentrate the analyte four fold. Then, Se(IV) and Se(VI) were separated in an anion separator column (Dionex 30589) using $0.008 \text{ mol.l}^{-1} \text{ Na}_2\text{CO}_3$ eluent. Se(IV) and Se(VI) were determined separately by ETAAS connected to the ion chromatograph by an interface. The use of this specific selenium detector allowed the analysis of river water, since anions such as Cl^- , NO_3^- , SO_4^{2-} , and PO_4^{3-} did not interfere. The detection limit was 20 ng.l^{-1} for Se(IV) and Se(VI).

Roden and Tallman [70] coupled the ion chromatography hydride generation-cold trapping-graphite furnace atomic adsorption spectrometry (IC-HG-CT-ETAAS) to determine Se(IV) and Se(VI) in groundwater. The samples were run through a column packed with XAS-8-resin (amberlite), which was freshly prepared for each sample, by passing 60 ml of KOH (pH 12-13) through the column followed by 60 ml of HCl (pH 1.6 to 1.8). Then, the sample was passed through the column to eliminate organic interferents, and the eluate (50 ml of HCl, pH 1.6 to 1.8) analyzed by HG-CT-ETAAS. Se(IV) was determined directly; but Se(VI) required a reduction step [54,55] and was determined by taking the difference between total selenium and selenium (IV).

McCarthy *et al.* [77] used a gradient elution, first with 0.005 mol.l^{-1} ammonium acetate (AAC)/ 0.002 mol.l^{-1} ammonium dihydrogen phosphate (ADP) at pH 4.6 and then with 0.08 mol.l^{-1} ADP at pH 6.9 to separate Se(IV), Se(VI), As(III) and As(V) in an anion-exchange column (Nucleosil-NH $(\text{CH}_3)_2$ on a silica support). The eluate was introduced to the ICP-AES by an interface, connecting the HPLC with the cross-flow nebulizer. The high detection limit of 140 ng for Se(IV) and 91 ng for Se(VI) did not allow the method to be applied to real samples.

Laborda *et al.* [78] used a cross-flow nebulizer and a thermospray vaporizer in their work on the interface between HPLC and ICP-AES, and proposed the use of organic solvents, such as methanol, methanol:water mixtures and 0.08 mol.l⁻¹ ammonium citrate, as eluent. These authors concluded that thermospray vaporizer provides three-fold higher sensitivity and that the fraction of methanol in the methanol/water mixtures introduced into the plasma can be as high as 75%, compared with 25% for the cross-flow nebulizer. They determined (CH₃)₃Se⁺, SeO₃²⁻ and SeO₄²⁻.

Recently, the same authors [79] separated trimethylselenium, selenite and selenate on an anion-exchange column (Nucleosil 100 SB) using 0.01 mol.l⁻¹ ammonium citrate eluent at pH 3 and 7. The selenium species were determined by ETAAS. The negative interference, produced by citrate, was avoided by adding 200 µg of nickel and 50 µg of magnesium nitrate. The detection limits were 1.67, 1.27 and 0.76 ng of Se for (CH₃)₃Se⁺, SeO₃²⁻ and SeO₄²⁻, respectively. The method was applied to water and urine.

The separation of Se-amino acids and inorganic Se-species has also been applied for the first time (Fig.7).

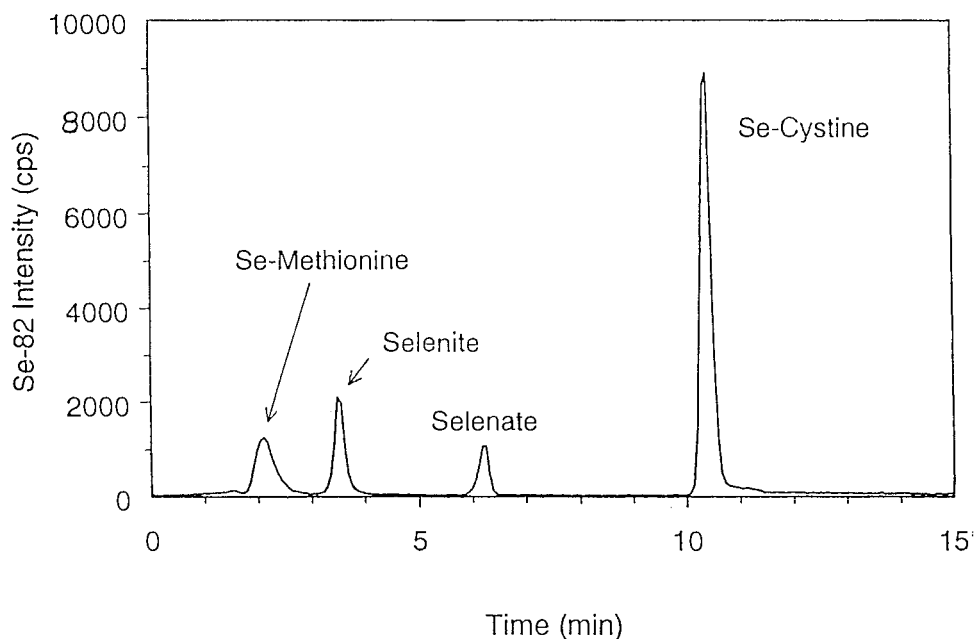


Figure 7: Ion chromatography separation of Se amino acids and inorganic Se species by HPLC-ICP-MS. Separation in a Dionex AS4A column, for a concentration of Se-amino acids of 1 and 5 mg.l⁻¹, respectively (reproduced with permission, adapted from [109])

10.5.4.2 Gas chromatography

The most common Se volatile compounds are dimethylselenide (Me_2Se) and dimethyldiselenide (Me_2Se_2). There is a wide variety of combinations of gas chromatography and detectors, such as GC/AAS, GC/ETAAS, GC/FPD, GC/AED, GC/SCD and GC/CTI/FA for the determination of these species.

10.5.4.2.1 Air samples

Volatile organoselenide species are sampled in air by cryogenic trapping: the air samples are cooled to liquid nitrogen temperature. The volatile selenides thus collected are thermally desorbed from the trap and transferred to an U-tube filled with the stationary phase and immersed in liquid nitrogen. Heating the tube drives the species to the detector [81]. Fig.8 depicts a chromatogram of DMSe and DMDSe trapped from air in a cold system using an atomic fluorescence detector.

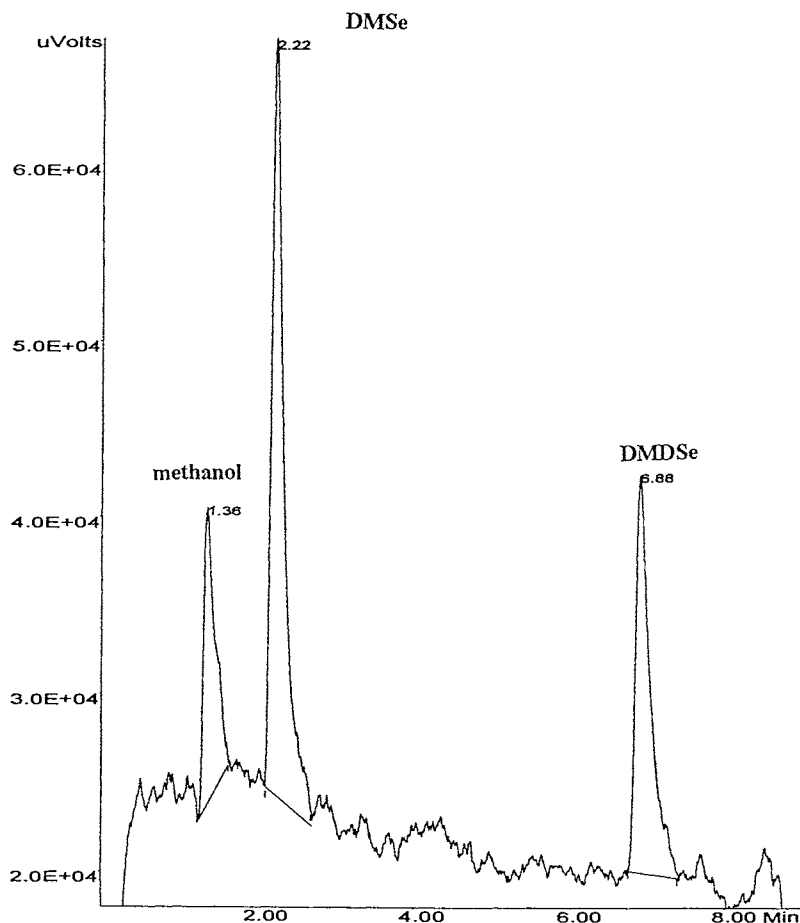


Figure 8: Volatile Se species detected by CT/QFAAS in the Gironde Estuary, France [110]

The AAS detection methods used include flame atomisation, quartz tube furnace, and graphite furnace.

The interface between the gas chromatograph and the AAS detector is usually made of a polytetrafluoroethylene (PTFE), nickel, or glass lined stainless steel tube which is heated electrothermally [82,83].

The column packing materials used for separation are 20% Carbowax [7], 10% OV-101 [90] or 10% polymetaphenylether [81] as the stationary phase, and Chromosorb W 60/80 mesh as the support [82,83].

Argon or nitrogen are normally the carrier gases. Hydrogen at 10% has a beneficial effect on the atomisation efficiency of selenium compounds.

The sensitivity of the GC-AAS coupling is affected by the injector, detector and chromatograph temperature. At low temperature the species tend to adsorb onto the injector, and excessive temperature leads to thermal decomposition of the compound. Injector and detector temperatures of 100°C and 160°C, respectively, are satisfactory [81].

The detection limits of GC-ETAAS [81] are about 0.2-0.3 ng.m⁻³ for Me₂Se, Me₂Se₂, and Et₂Se in air samples.

An atomic absorption technique using a silica furnace gave a detection limit of 0.1 mg.l⁻¹ with efficient separation and very high specificity [84].

GC/AAS was also applied to study the transformation of inorganic selenium to methylated selenium species in laboratory experiments, *e.g.* with animals such as mice [85]. After administration of selenite and selenocystine in drinking water, Me₂Se was the predominant resultant species, however, when selenomethionine was administered, Me₂Se as well as Me₂Se₂ and an unknown product were exhaled [81]. These results represent a step forward in understanding the metabolism and biotransformation of these compounds.

These methods have not all been validated with other analytical techniques or with reference materials.

10.5.4.2.2 Water samples

Me₂Se has been postulated to be present in seawater. The volatile species in water samples were collected and concentrated using a purge and trap method. The samples were transferred from a PTFE vessel through a membrane filter (to remove algae cells from the sample). Once loaded, the sample was driven to a glass bubbling chamber for purging. Volatile compounds were stripped from the sample and trapped in a cold trap immersed in liquid nitrogen and coupled to the GC separation column. When the purging was complete, the cold trap was heated and the species desorbed sequentially to the detector [86].

A first analysis carried out by a GC/FPD system revealed several peaks from sulphur compounds and an unknown peak probably due to selenium. The later split into a Me₂Se and an unknown sulphur compound peak on analysis by GC-AED. The detection limit was 50 pg.l⁻¹ for Me₂Se [86].

Volatile compounds of Se₂(CH₃)₂ in seawater have been for the first time detected by CT/AAS (Fig.9).

10.5.4.2.3 Soil samples

Samples of volatile Se compounds have been obtained from soil by spiking it with inorganic selenium (IV) and incubating at 30°C for three days. After incubation, the volatile species produced by biomethylation of the added Se(IV) were swept into hexane and analysed by GC-ETAAS. The detection limits for natural samples were at the picogram level (5 pg for Me₂Se and Et₂Se and 20 ng for Me₂Se₂) but this technique has not yet been validated [87].

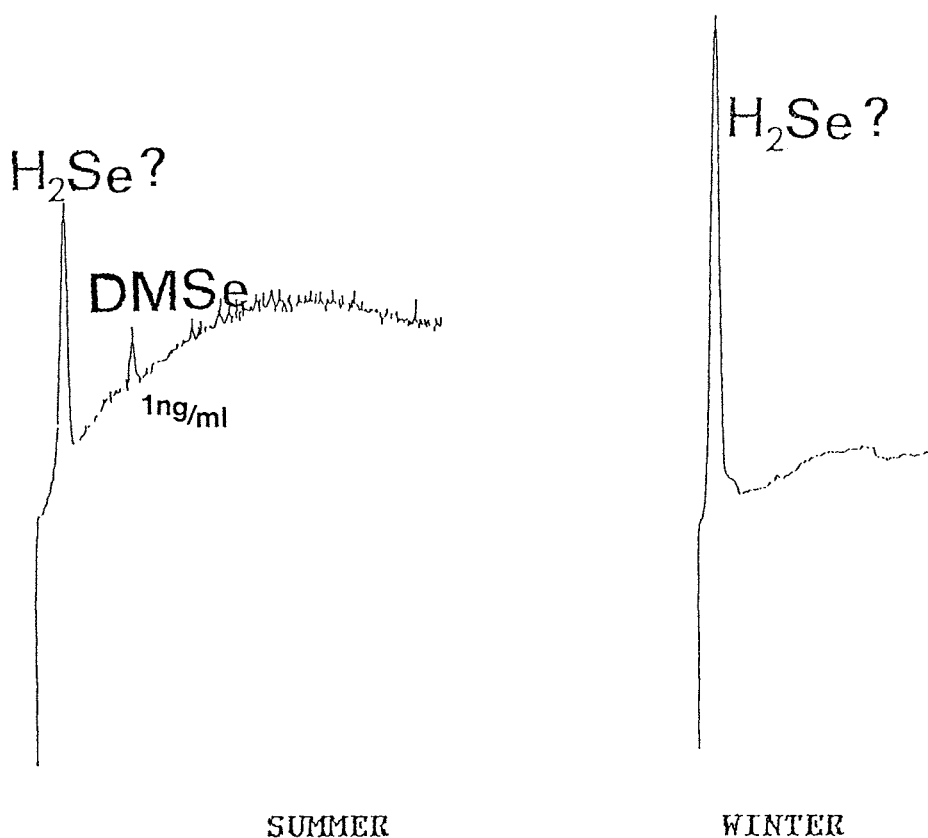


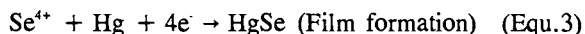
Figure 9: Speciation of volatile Se compounds by GC using an atomic fluorescence detector. Concentration of 80 pg of DMSe and DMDSe (the methanol peak results from a spectral interference of the solvent) [110]

10.5.4.2.4 Biological samples

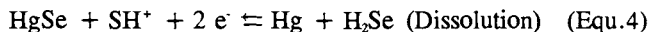
The development of a sulphur chemiluminescence detector (SCD) has permitted the detection of the volatile Se species in bacterial and fungal cultures [88]. The interest in studying these organisms is their ability to biomethylate inorganic selenium administered to the cultures, which is converted into organic selenium. The technique makes use of a capillary GC coupled to fluorine-induced chemiluminescence detection. Fluorine is produced in line by a high frequency electrical discharge of a stream of SF_6 . Fluorine reacts with the analyte in the gaseous phase to form HF in an excited vibration state, and the CH_2Se^* molecule which is responsible of luminescence. The method requires no sample derivatization and the detection limits for methylselenides are in the low pg range.

10.5.5 Electrochemical methods

The most common electrochemical methods of selenium speciation in natural waters are: differential pulse polarography (DPP) and differential pulse cathodic stripping voltammetry (DPCSV), Fig.10. Both are based on the electrochemical determination of Se(IV), which can be reduced at a mercury electrode in two discrete steps [89,91]:



This reaction is irreversible. Se(IV) can be accumulated at an electrode surface and the enriched selenide layer can be stripped cathodically by the reversible process:



Alam *et al.* [92] determined Se(IV) in HCl and HClO_4 media by polarography at a dropping mercury electrode. They achieved a detection limit of $10 \mu\text{g.l}^{-1}$. The use of 2-12 min selenium accumulation before cathodic stripping yielded at detection limit of 0.1 to $2 \mu\text{g.l}^{-1}$. The interference of Pb(II) can be prevented by the addition of EDTA.

Howard *et al.* [93] proposed another electrochemical method to determine Se(IV) by differential pulse polarography (DPP) of the 4-chloro-o-phenylenediamine piarselenol, which gives a reduction peak at 0.11 V vs SCE at pH 2.5 (Fig.11). Interferences from Cr(VI), Cu(II), Mo(VI), Ni(II), Zn(II), Te(IV) and V(V) can be overcome by sample treatment on a Chelex-100 resin column and subsequent determination of Se(IV) by DPP. The method was applied to fresh, estuarine and sea waters, and had a detection limit of $0.4 \mu\text{g.l}^{-1}$.

Campanella *et al.* [94] used DPP and Differential Pulse Cathodic Stripping Voltammetry (DPCSV) to determine Se(IV) in natural waters. Two mechanisms for the reaction at a mercury electrode are proposed: a) Formation of Se^0 and further reduction to H_2Se and b) formation of a HgSe film, which then dissolves as mercury is reduced. The sample was filtered to separate the undissolved and soluble phases. The solid fraction was wet digested to obtain soluble selenium compounds. To quantify these, the subsample was divided in different fractions and all of them were treated on Chelex-100 columns to obtain clean blanks. Since selenourea was adsorbed onto the column, UV irradiation [95-97] was essential to destroy organo-compounds. Se(IV) was measured directly in one aliquot obtained from a chelex column, and another aliquot was treated with HCl in order to reduce Se(VI) to Se(IV), so Se(VI) was determined by taking the difference between total selenium and Se(IV). The detection limits are $100 \mu\text{g.l}^{-1}$ for DPP and $0.04 \mu\text{g.l}^{-1}$ for DPCSV.

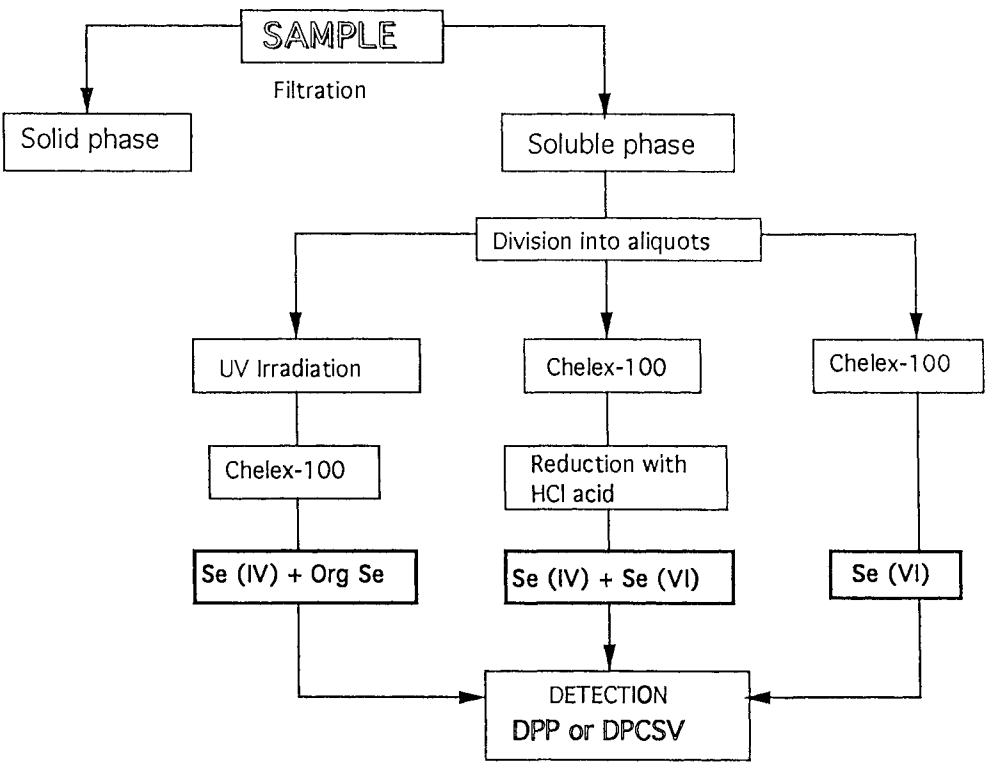


Figure 10: Electrochemical methods used in Se speciation

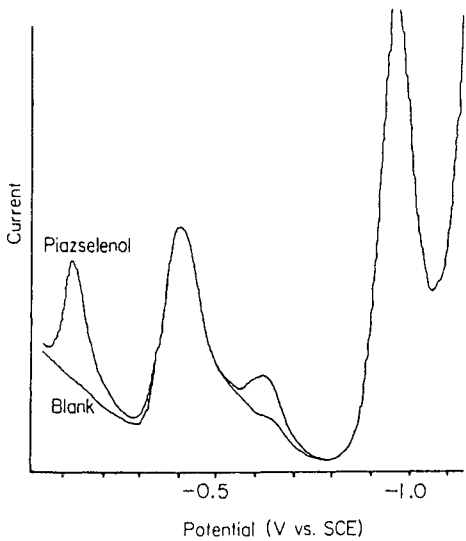


Figure 11: Electrochemical detection of Se(IV) .Polarogram of 4-VI-PDA piazselenol at pH 2.5 [93]

10.5.6 Neutron activation analysis (NAA)

Neutron activation analysis (NAA) is not very sensitive for Se speciation in environmental samples. As with the atomic spectroscopy techniques, the selenium species must be separated before they can be determined. Low concentrations of selenium are usually determined using preconcentration techniques. ^{76}Se and ^{78}Se are the radioisotopes currently used in activation analysis. ^{76}Se provides the highest sensitivity, by its very short half-life (17.5 s), is used only in instrumental neutron-activation analysis. ^{78}Se is used more often because its long half-life (120.4 days) allows chemical separation, although long activation times are required.

Enrichment of selenium is always necessary with this technique. The three selenium preconcentration methods that have been used are: selective extraction [98, 99], non selective extraction [100] and freeze-drying [101,102].

Massee et al. [103,104]determined Se(IV) and Se(VI) by NAA and reported a detection limit of $10\text{ }\mu\text{g.l}^{-1}$. The main steps in this method were: a) reduction of Se(VI) to Se(IV) by refluxing after addition of concentrated HCl, b) reduction of Se(IV) to elemental selenium with ascorbic acid, c) adsorption of elemental selenium by activated charcoal, and d) determination of selenium by NAA. Obviously, to determine Se(IV) the first step is omitted. This method was applied to sea and fresh water.

Orvini *et al.* [105] proposed a speciation methods for the determination of selenium in polluted river water, consisting of the following steps: a) filtration, b) collection on an anion-exchange resin for determination of Se(IV) and Se(VI) in suspended and colloidal compounds, c) reduction of Se(VI) to Se(IV) in HCl medium, d) selective reduction of Se(IV) to elemental selenium, e) charcoal adsorption and f) selenium determination by NAA. They found an significant amount of selenium in the colloidal fraction.

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11.

Antimony speciation in water

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In comparison with other metals few papers have been published on the selective determination of antimony species, reflecting the lack of knowledge of its environmental chemistry. Nevertheless, interest in the determination of this toxic element, even at trace level, has grown over the last few decades as a consequence of an increment in its industrial applications.

Nowadays antimony has a number of industrial applications such as the use of Sb-Ga and Sb-In alloys in semiconductors that are promising materials in the manufacture of high speed computer chips [1] and for optical information memories operated by laser signals (compact discs, digital optical recording devices, *etc.*). Antimony is also used in certain therapeutic agents against major tropical parasitic diseases, although in recent years it has been increasingly replaced by other agents, mainly antibiotics. Recently it has been observed that ammonium-5-tungsto-2-antimoniate is an effective inhibitor of reverse transcriptase and therefore of possible utility against the AIDS virus [2].

Antimony is released into the environment from natural processes (*e.g.* the weathering of rocks and soil runoff), mining effluents and industry (*e.g.* glasses, semiconductors, dyestuffs, ceramics and fire retardants). In the particular case of antimony oxide the main polluters are the smelting industry and the fossil fuel power stations.

Around $3.8 \cdot 10^{10}$ g/year of antimony are released into the environment as a result of human activities, and it may be transported long distances through the atmosphere before accumulating in soil, lichens, *etc.* For these reasons, antimony and its compounds are considered to be pollutants of priority interest by the US Environmental Protection Agency (EPA).

Four species of antimony have been identified in natural waters: Sb(III), Sb(V), monomethylstibonic acid and dimethylstibinic acid. In oxygenated waters the predominant species is Sb(V), although higher concentrations of Sb(III) and methylated compounds than would be expected from thermodynamic calculations have been detected. This could be attributed to microbial activity, which is suspected to favour such a transformation as a detoxification mechanism since the methylated species of antimony are less toxic than the inorganic ones.

In view of the high toxicity of antimony (European Community Standards set the maximum admissible level of antimony in drinking and surface water at $10 \mu\text{g.l}^{-1}$), it is essential to develop sensitive analytical methods for its determination in natural samples. However, as in many other instances, it is not sufficient to determine the total concentration of antimony, and the concentration of the different species present in the sample must be known, since it affects: a) toxicity (Sb(III) is more toxic than Sb(V) [3] and inorganic oxyanions are normally more toxic than organic compounds [4]), b) metabolism: Sb(V) is eliminated mainly in urine and Sb(III) in faeces [5], and c) reactivity, *e.g.* stibine is generated more efficiently from Sb(III) than from Sb(V) in antimony hydride generation [6].

The purpose of this chapter is to summarize and to discuss the existing analytical methods for antimony speciation in water, in order to provide easy access to this topic to all interested researchers.

11.1 Stability of antimony species

The relative proportions of the different species of an analyte may change significantly during the long-term storage of both natural samples and synthetic solutions. The reasons are various and include the effects of light, temperature, pH, microbial activity and container material.

Long-term storage is, however, frequently necessary, *e.g.* in interlaboratory comparisons or the preparation of calibrant solutions. Therefore, before discussing the analytical methods for antimony speciation, we will briefly look at the results which several research groups have obtained on antimony species stability in aqueous solutions.

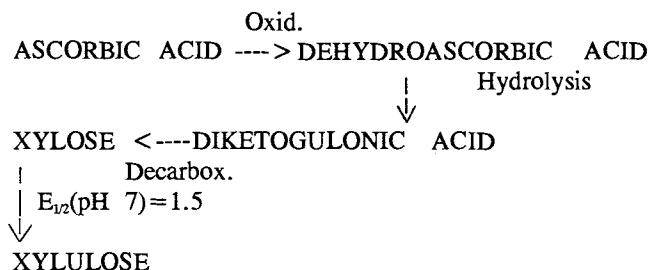
The results in this respect are conflicting. Sun *et al.* [7] reported that at the ng.l^{-1} level Sb(III) added to lake water or waste water was not detected within six hours, probably because it was oxidized to Sb(V) by oxidants present in the samples. When up to 1% (w/v) of tartaric acid was added to the samples, the concentration of Sb(III) remained unaltered for at least five days. Sb(V) concentrations at the $2 \mu\text{g.l}^{-1}$ level remained constant for five days, and then underwent a slight decrease which was not prevented by the addition of tartaric acid. The authors suggested that the decrease in Sb(V) concentration was due to the adsorption of Sb(V) onto the container walls, but no mention was made of the container material.

Andreae *et al.* [6] used potassium antimonyl tartrate to obtain Sb(III) calibrant solutions and reported that at the $1000 \mu\text{g.l}^{-1}$ level these solutions were highly stable. In some low concentration solutions Sb(III) was oxidized to Sb(V) within half an hour and in other cases the concentration of Sb(III) remained constant for several months. It was suggested that the quality of the water used to make the dilutions was responsible for the oxidation of Sb(III), the presence of small amounts of oxidants could produce the Sb(III) oxidation. Andreae [8] recommended the storage of filtered samples by deep freezing when the analysis is not performed immediately after collection.

An extensive study on antimony species stability has been carried out by de la Calle *et al.* [9]. Eight solutions containing $25 \mu\text{g.l}^{-1}$ Sb(III) and $25 \mu\text{g.l}^{-1}$ Sb(V) were prepared in four different media: two in aqueous medium, two in 1% (v/v) lactic acid medium, two in 1% (w/v) citric acid medium and two in 1% (w/v) ascorbic acid medium.

Lactic and citric acid media were chosen because both these acids form complexes with antimony [10,11], and ascorbic acid medium was included because of its antioxidant properties. The solutions were stored in polyethylene bottles. Four (one of each medium) were kept in the refrigerator at 0-4 °C and the other four at room temperature. The stability study was carried out over a period of one year. Sb(III) was determined by hydride generation/atomic absorption spectrometry (HG-AAS) and total antimony by electrothermal AAS (ETAAS). Sb(V) was calculated as the difference between total antimony and Sb(III). This study showed that Sb(III) (at 25 $\mu\text{g.l}^{-1}$) was stable at 0-4 °C for at least 12 months in aqueous solution, in 1% (w/v) lactic acid and 1% (w/v) citric acid media, when the solutions were kept in polyethylene flasks. Surprisingly, a possible oxidation of Sb(III) to Sb(V) took place after one month in 1% (w/v) ascorbic acid medium even at a temperature of 0-4 °C. Furthermore, solutions stored in this medium became yellow (0-4 °C) or brown (room temperature) and had a "sugary" smell (stronger at room temperature than at 0-4 °C). The solutions prepared in the other media remained colourless and odourless.

This behaviour could be explained by the participation of ascorbic acid in the following reaction



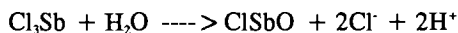
The presence of xylulose, commonly called "light yellow syrup", would explain the colour and smell of the solutions prepared in ascorbic acid medium. Considering the $E_{1/2}$ of the last of the above reactions, the reduction of xylose to xylulose could produce the oxidation of Sb(III) to Sb(V). Further studies are, however, required to confirm this hypothesis.

At room temperature the Sb(III) concentration decreased sharply after one month in lactic acid medium and after three months in aqueous medium. More promising results were obtained in citric acid medium although it did not completely prevent a decrease in the Sb(III) concentration.

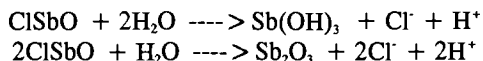
The total antimony concentration remained constant for 12 months at 0-4 °C and at room temperature. The results of De la Calle *et al.* suggested that polyethylene flasks were adequate for the storage of antimony solutions since the analyte was not adsorbed onto the vessel walls.

This high stability of total antimony is in disagreement with the results reported by Smith [12] who studied the stability of antimony solutions at pH values between 1 and 11 obtained by the addition of HCl and NaOH. After 24 h of storage a significant decrease in antimony concentration was detected in the pH 2-8 range, and it was more pronounced in the 10 mg.l^{-1} solutions than in the 1 mg.l^{-1} solutions. Both lower and higher pH than 2-8 allowed a good stability to be achieved. The addition of HCl

to the solutions might be responsible for the instability observed, since the equilibrium:



is shifted to the right by the addition of water, leading to the precipitation of $\text{Sb}(\text{OH})_3$ or hydrated Sb_2O_3 :



This hydrolysis can be avoided by the addition of sufficient HCl , which would explain the high stability at $\text{pH} < 1$, or by the addition of tartaric acid to form the antimonyl tartrate cation. In strongly basic media the predominant species is SbO_2^- , which is soluble in water.

De la Calle *et al.* [9] reported that synthetic $\text{Sb}(\text{III})$ and $\text{Sb}(\text{V})$ solutions prepared from potassium antimonyl tartrate and potassium pyroantimonate were stable for at least 12 months when stored in polyethylene flasks at $0-4^\circ\text{C}$, and there was no need to add stabilizing agents, such as organic acids, which may cause interference if, *e.g.* electroanalytical techniques are to be used. This is an advantage for the preparation of calibrant solutions, in method validation and interlaboratory comparisons. It was assumed that the decrease in $\text{Sb}(\text{III})$ concentration was due to oxidation to $\text{Sb}(\text{V})$; nevertheless, further studies are required to check whether this is the only a transformation or whether $\text{Sb}(\text{III})$ participates in reactions forming organoantimony compounds such as monomethylstibonic acid and dimethylstibinic acid.

11.2 Analytical methods for the determination of inorganic antimony

11.2.1 Liquid-liquid extraction

The ability of certain complexing agents to react with the different species of an analyte, which is then selectively extracted under defined conditions, has been widely used for the speciation of most metals. Liquid-liquid extraction also removes the analyte from the rest of the matrix and in most instances enables good preconcentration factors, which are of great interest considering the very low concentrations of some metals in natural samples.

11.2.1.1 Spectrophotometric determination

Several methods have been described for the spectrophotometric determination of antimony. These methods are based on the extraction of $\text{Sb}(\text{V})$ as SbCl_6^- with cationic dyes such as rhodamine B [13], crystal violet [14] and thiazolyl blue [15]; or of $\text{Sb}(\text{III})$ complexed with ethylxanthates [16], butylrhodamine B [17], OO' -diethyldithiophosphate [18] or other reagents [19]. However, they have not been applied to the differential determination of $\text{Sb}(\text{III})$ and $\text{Sb}(\text{V})$ because of their lack of selectivity for one of the two antimony oxidation states. The methods based on the extraction of $\text{Sb}(\text{V})$ required the addition of an oxidant such as NaNO_2 , to ensure that all antimony is present as $\text{Sb}(\text{V})$ and the methods based on the extraction of $\text{Sb}(\text{III})$ would require the addition of a reductant, such as KI or ascorbic acid.

Sato [20] described the speciation of Sb(III) and Sb(V) based on the different rates of reaction of the two oxidation states with mandelic acid and subsequent formation of an ion pair with malachite green that can be extracted into chlorobenzene. Sb(III) readily reacts with mandelic acid at room temperature and pH 2.2 to 3.5 to form an anionic complex, while Sb(V) requires a temperature of 45 °C for 15 minutes to obtain a complete reaction. Although a previous paper of Sato *et al.* [21] proposed the use of phosphate buffer to adjust the pH, the buffering ability of the α -hydroxyacids made this step unnecessary and it was omitted in later works [20], Figure 1.

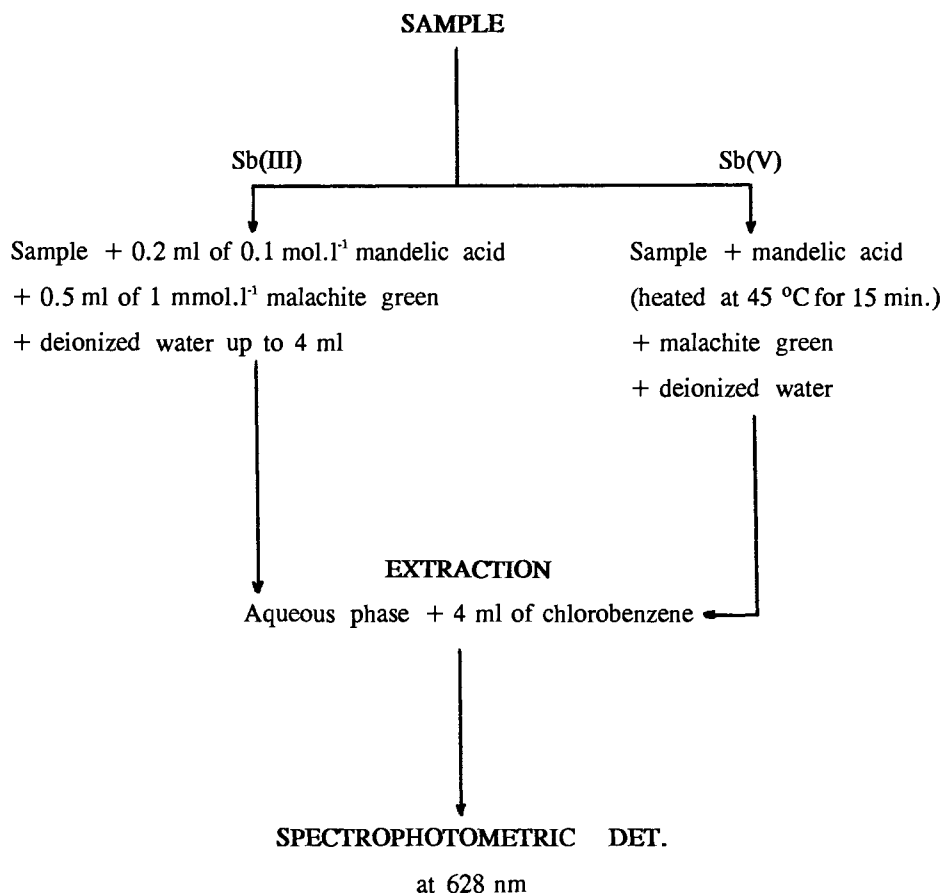


Figure 1: Sb(III) and Sb(V) selective determination with spectrophotometric detection, following the method proposed by Sato [20].

Sato and Uchikawa [22] carried out the speciation of Sb(III) and Sb(V) based on the formation of a complex with 2-hydroxy-4-methylpentanoic acid. This acid forms a complex with Sb(III) instantaneously at pH 3.0 and with Sb(V) in 15 min. at 45 °C. The complexes can be extracted into chlorobenzene in the presence of malachite green, but the Sb(III) complex is not extracted in the presence of citrate, thus enabling Sb(V) to be determined in the presence of Sb(III). Total antimony can be determined without the addition of citrate, Figure 2. The great advantage of this method is that it enables the direct determination of Sb(V), while most other methods require Sb(V) to be determined as the difference between total antimony and Sb(III), with the associated problems of poor precision and accuracy.

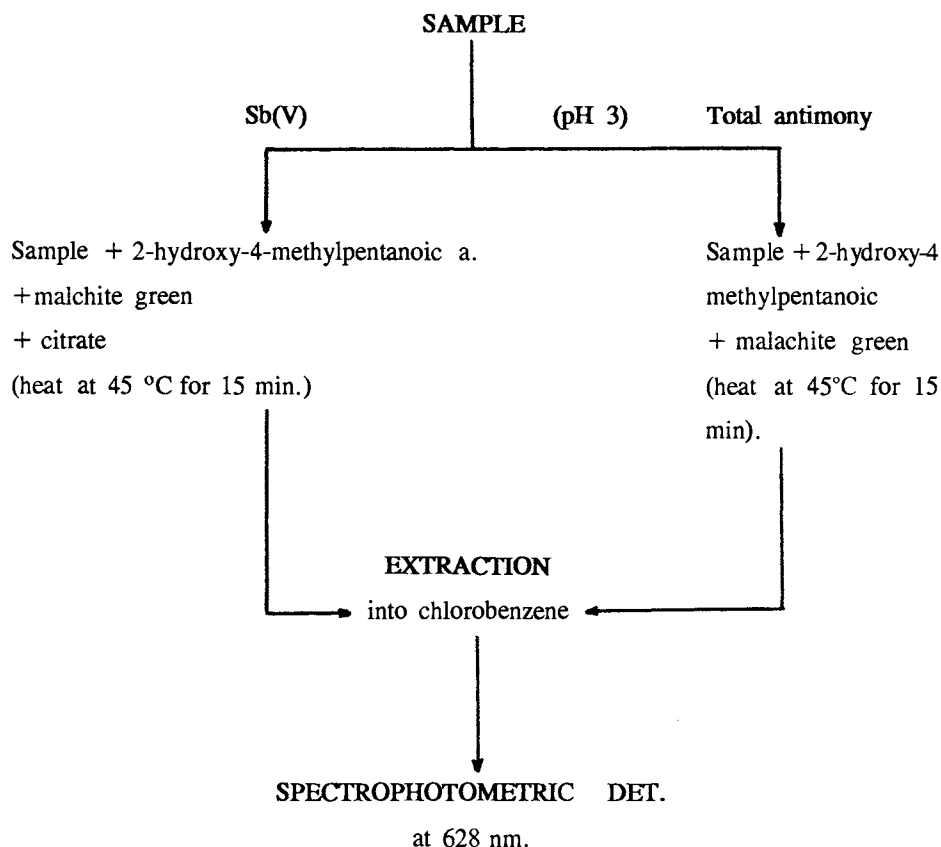


Figure 2: Sb(III) and Sb(V) speciation procedure with spectrophotometric detection, proposed by Sato and Uchikawa [22].

Yonehara *et al.* [23] speciated Sb(III) and Sb(V) by an indirect method based on the determination of Sb(III) by reaction with excess Cr(VI). The excess Cr(VI) was quantified by treatment with diphenylcarbazide and measurement of the complex absorbance at 540 nm. Total antimony was calculated by reducing Sb(V) to Sb(III) with Na_2SO_3 in HCl. Sb(V) is calculated as the difference between total antimony and Sb(III) concentrations, Figure 3.

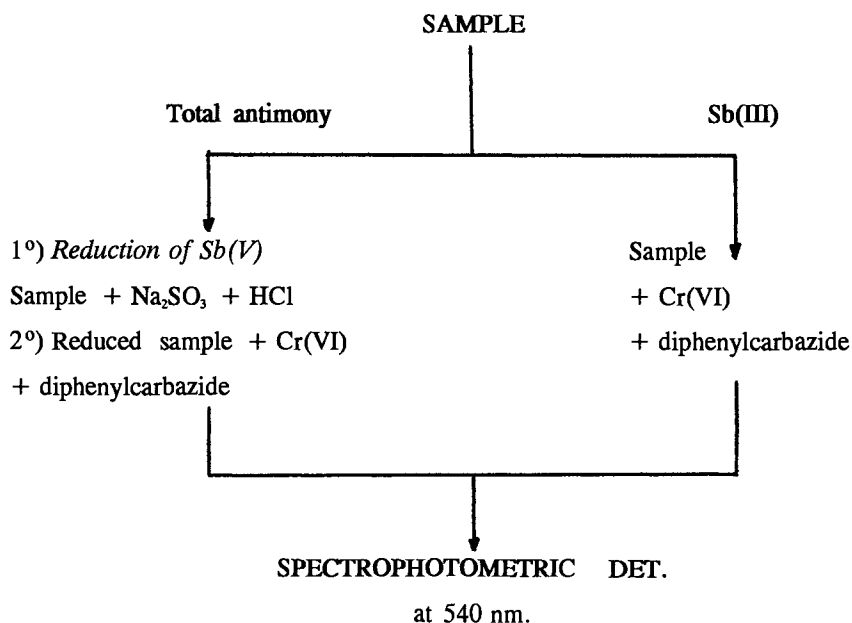


Figure 3: Sb(III) and Sb(V) speciation procedure with spectrophotometric detection, proposed by Yonehara *et al.* [23].

All the above-mentioned methods based on the spectrophotometric determination of Sb(III) and/or Sb(V) allow the determination of antimony only at the mg.l^{-1} level, which in most instances exceeds the concentration in natural samples, where even the total antimony concentration is normally at the ng.l^{-1} level.

11.2.1.2 Detection by electrothermal atomic absorption spectrometry

Some authors have suggested that the absence of a nebulization system in the ETAAS should make it possible to analyze viscous extracts, and that the total elimination of organic solvents in the drying step would eliminate the background in the atomization step. It has been also proposed that in the ashing step most of the complexes extracted into organic solvent decompose into stable inorganic compounds. On the basis of all this, it might be thought that the determination of metals by

ETAAS when present in an organic solvent would not present any additional difficulty with respect to the determination in aqueous solutions. However several drawbacks are common to these kind of analysis, mainly because of the ability of organic solvents to spread on several materials such as graphite, plastic, *etc.* The properties of organic solvents to penetrate into the graphite and to slide on the graphite surface, are the main responsible factors for high irreproducibility, distorted signals, low sensitivity and non linear calibration curves. The spreading ability of organic solvents also produces a sampling error of 7% while for aqueous solutions it is only 1.5%. The amount of extract remaining in the pipette can be as much as 30%.

In general, the sensitivity is lower for organic solvents than for aqueous solutions for a variety of reasons, such as the dispersion of the solvent on the tube surface, the nature of the organic solvent and of the complexing agent, and the volatility of the extracted compound, which in some cases sublimates thus causing incomplete atomization, *etc.*

Despite these disadvantages, liquid-liquid extraction with ETAAS determination of the metal is one of the analytical methods which is most widely applied to the antimony speciation. Some of them are described in the following section.

Sun *et al.* [24] carried out such speciation analysis by forming the complex Sb(III)-N-benzoyl-N-phenylhydroxylamine (BPHA), which is extractable into chloroform. Sb(III) can be quantitatively extracted from pH 6 to 2 mol.l⁻¹ HCl. Sb(V) is not extracted in this pH range, hence Sb(V) must be prereduced to Sb(III) with KI to determine the total concentration of antimony. Sb(V) is then determined as the difference between total antimony and Sb(III), Fig.4.

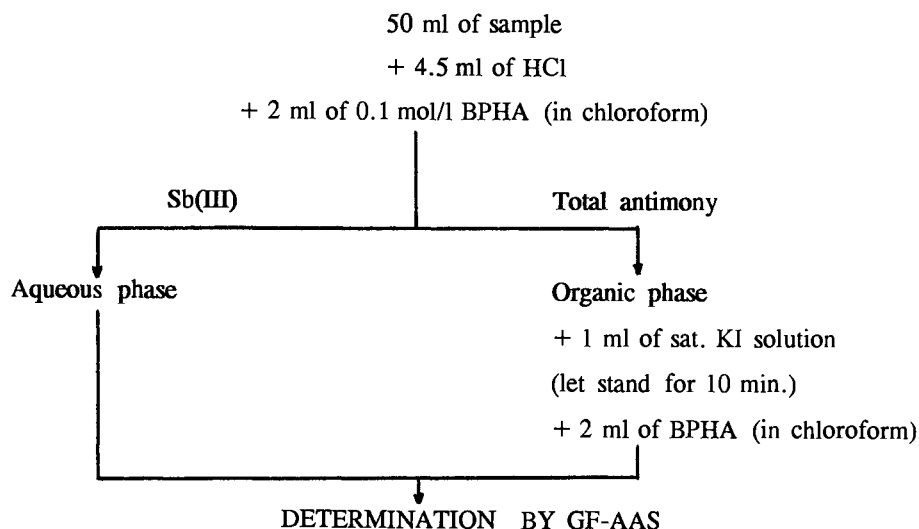


Figure 4: Sb(III) and Sb(V) speciation procedure by L-L extraction and GF-AAS detection proposed by Sun *et al.* [24].

A similar procedure was applied by Abassi [25] who used N-(4-metoxypheyl)-2-phenylacrylohydroxamic acid as the complexing agent and extracted the formed complex in chloroform.

De la Calle *et al.* [26], Fig.5, selectively determined Sb(III) and Sb(V) by forming a complex between Sb(III) and lactic acid, which is extractable with malachite green into chloroform. The formation of the [Sb(III)-lactic acid][malachite green]⁺ ion pair is immediate, and the ion pair is extracted into chloroform with 80% extraction efficiency by shaking for 15 s. The method proposed by de la Calle *et al.* has the drawback that Sb(V) remains in the aqueous phase and, in contrast to the results obtained by Sato [20] (with spectrophotometric detection), is not extracted into the organic phase even when Sb(V) and lactic acid are allowed to react at high temperature (45-50 °C) for as long as one hour. In further studies [10] the Sb(V) reacted immediately with lactic acid even at room temperature, so its non-extractability was probably because an ion pair with malachite green was not formed. Consequently Sb(V) had to be determined in the aqueous phase. It could not be determined as the difference between total antimony (after reduction with KI) and Sb(III) because the addition of KI degraded malachite green.

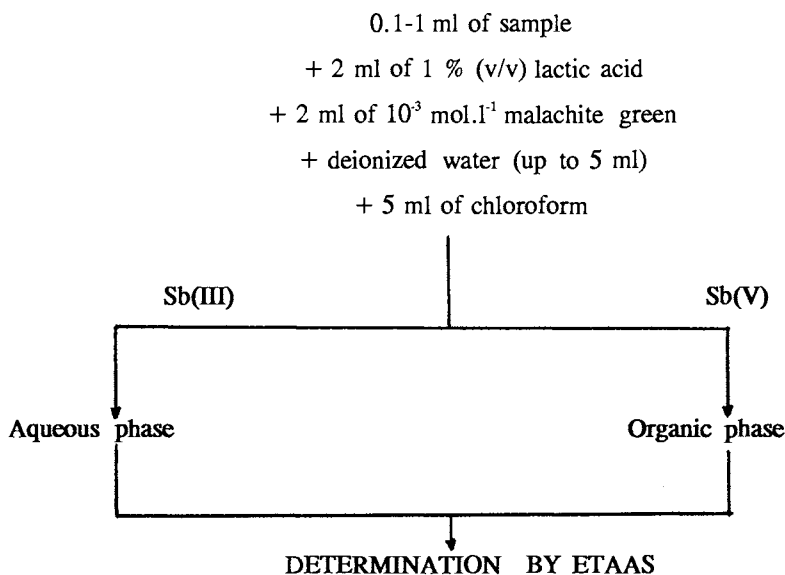


Figure 5: Sb(III) and Sb(V) selective determination by L-L extraction and ETAAS detection, following the method proposed by De la Calle *et al.* [26].

This behaviour limits the applicability of this method to drinking and surface water; if sea water has to be analyzed the determination of Sb(V) will require the use of a matrix modifier.

Nevertheless, the most widely applied system involves the formation of a complex with ammonium pyrrolidinedithiocarbamate (APDC) and several speciation procedures based on the use of this complexing agent have been described. Chung *et al.* [27] and Chi *et al.* [28] developed procedures in which Sb(III) was determined first. To do this, the APDC complex is formed and extracted at pH 5 into a chloroform/carbon tetrachloride mixture. Under these conditions Sb(V) remains in the aqueous phase. Total antimony was determined by the reduction of Sb(V) to Sb(III) with TiCl_3 and extraction of the complex at pH 0.3. The concentration of Sb(V) was calculated as the difference between total antimony and Sb(III), Fig.6.

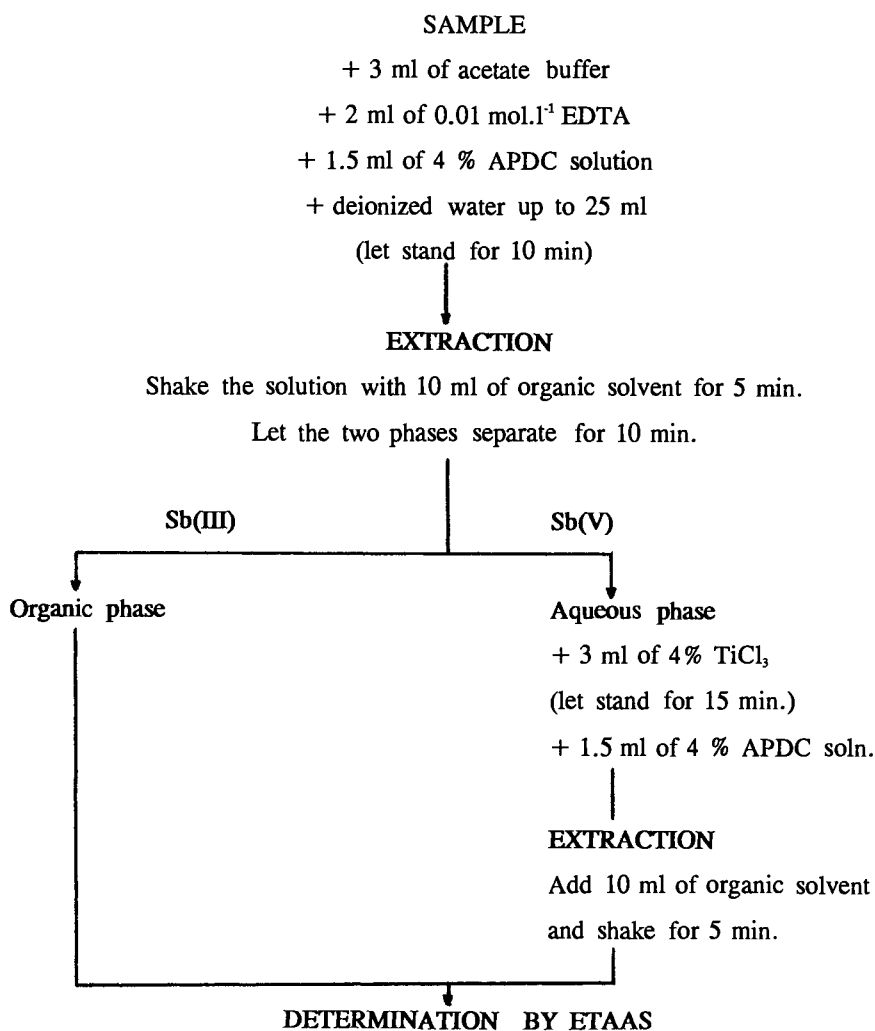


Figure 6: Sb(III) and Sb(V) selective determination by L-L extraction and ETAAS detection, following the method proposed by Chung *et al.* [27].

Subramanian and Meranguer [29] systematically studied the ammonium pyrrolidine-dithiocarbamate-MIBK system and concluded that Sb(III) and Sb(V) can be distinguished without reducing Sb(V) to Sb(III), simply by controlling the extraction pH. The authors studied the relationship between the pH of the aqueous solution prior to APDC addition and the percentage of extraction. The complex is completely extracted in only one extraction step over the pH 0.0-9.0 range for Sb(III), but no extraction of Sb(V) takes place at pH 2.5-10.0. Total antimony can be determined because both Sb(III) and Sb(V) are simultaneously extracted in the acidity range 0.3-1.0 mol.l⁻¹ HCl.

Sb(III) is extracted by shaking for 5 s; Sb(V) requires 5 min shaking for quantitative extraction from 0.5 mol.l⁻¹ HCl at 25 °C. If a 0.5 mol.l⁻¹ HCl solution containing Sb(V) and 1 % APDC is boiled for one minute and cooled to 25 °C, complete extraction only requires 30 s shaking. These results suggest that the complexation of Sb(V) with APDC is slower than that of Sb(III), probably because Sb(V) is present as an oxoanion highly stable in aqueous acid.

Subramanian and Meranguer also studied the stability of the extracted chelates. The Sb(III) chelate is stable for 2 months if the aqueous acidic phase is drained off and the MIBK phase is rinsed twice with high purity water and transferred into a clean dry flask. The stability may also be improved by back-extraction of the chelate from the MIBK into a smaller volume of acidified (pH 1.0) aqueous solution, Fig.7.

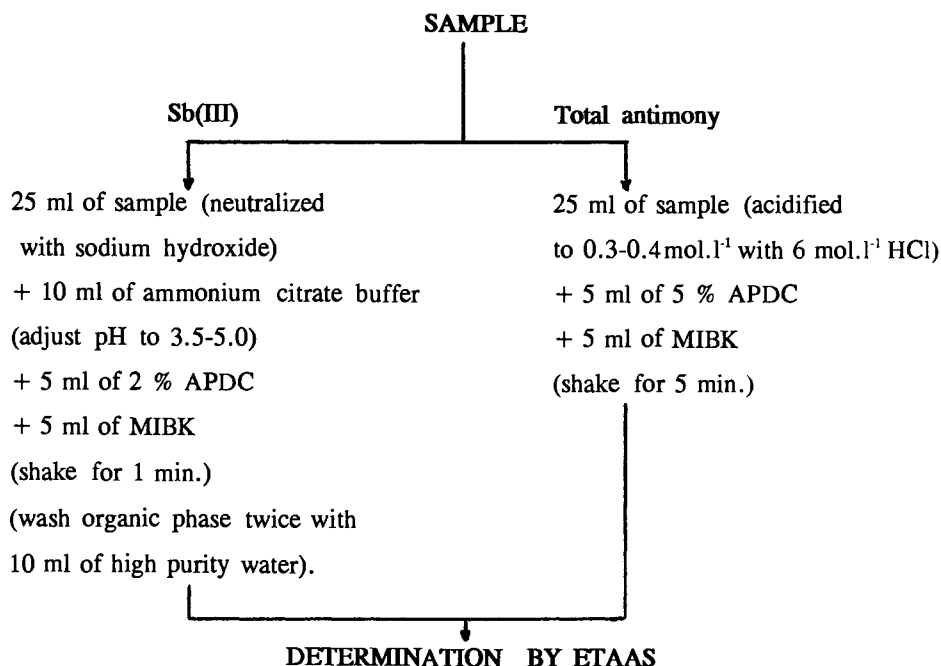


Figure 7: Sb(III) and Sb(V) speciation procedure by L-L extraction and ETAAS detection, proposed by Subramanian and Meranguer [29].

Iwamoto *et al.* [30], Fig.8, studied the interference by Sb(V) in the determination of Sb(III) using the ammonium pyrrolidindithiocarbamate (APDC)-MIBK or dichloromethane system. They concluded that Sb(V) is partially extracted at pH higher than 3.5 when APDC is added to a solution of Sb(V) of pH < 3. They also suggested that this behaviour was because at pH < 3 Sb(V) is mainly present as Sb(OH)₅, [31], which probably leads to the formation of a complex extractable in organic solvents with APDC; once the complex has been formed below pH 3, it is stable at pH 3 or higher. At pH > 3 the Sb(V) is present as Sb(OH)₆⁻, which does not form an extractable complex. There was also a decrease in the extraction efficiency of Sb(III) when Sb(V) coming from a solution of potassium antimonyl tartrate, was present in the medium. To avoid this effect the authors proposed the addition of an auxiliary complexing agent (tartrate or citrate), although the mechanism of such interference is not completely established.

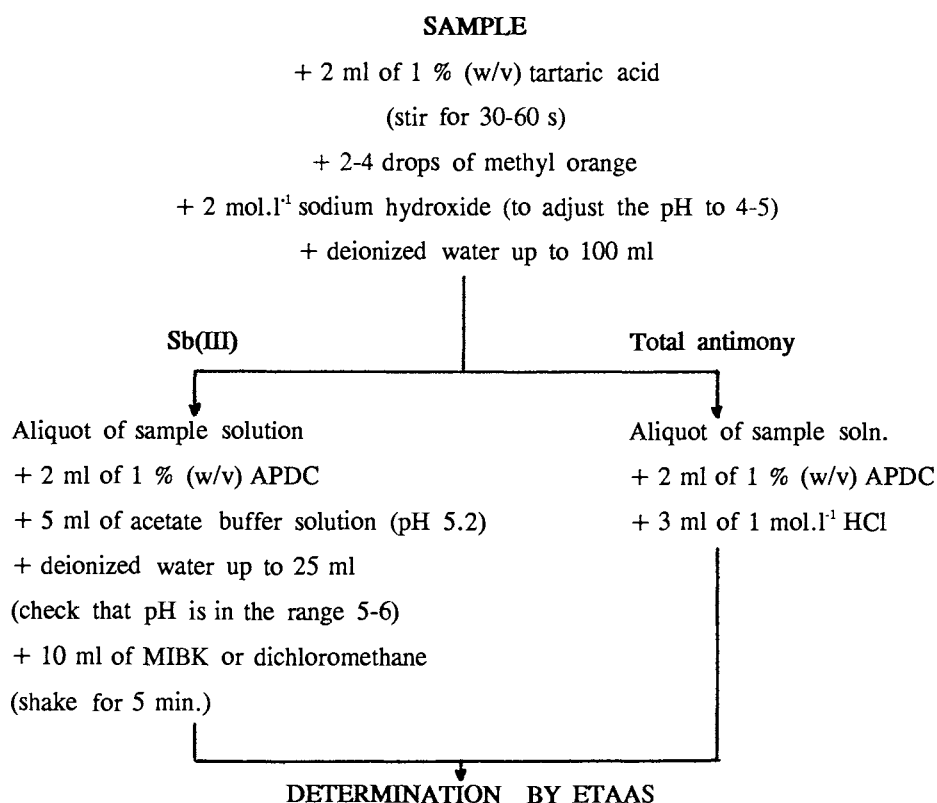


Figure 8: Sb(III) and Sb(V) speciation procedure by L-L extraction and GF-AAS detection, proposed by Iwamoto *et al.* [30].

Antimony and APDC complexes have been also used for the selective determination of Sb(V) and Sb(III) in combination with techniques other than ETAAS, such as electroanalysis (cyclic voltammetry [32]) and neutron activation [33]. Metzger and Braun [32] selectively determined Sb(III) and Sb(V) by cyclic voltammetry as follows. Total antimony was determined by treating antimony solutions with $K_2Cr_2O_7$, HCl and ascorbic acid solutions followed by voltammetry with repeated polarization cycles. To selectively determine Sb(III), the pH was adjusted to 4.5 to form a Sb(III)-APDC complex, which was then extracted into MIBK and back-extracted into a $K_2Cr_2O_7$ solution. HCl and ascorbic acid were then added and the determination made as for total antimony. Sb(V) was determined by difference or directly by voltammetry after extraction of Sb(III) into chloroform as a complex with N-phenylbenzohydroxamic acid and treatment of the aqueous phase as for total antimony determination.

For Sb(III) and Sb(V) speciation by neutron activation, Mock and Wai [33] selectively extracted the Sb(III)-APDC complex formed in the pH 3.5-5.5 range. Sb(V) was determined by reduction to Sb(III) with $Na_2S_2O_3$ and KI and extraction of the complex formed with APDC, but now adjusting the pH to 1.

Generally the methods based on liquid-liquid extraction and ETAAS determination are time consuming, but the limit of detection achieved is usually low enough to determine antimony in natural samples, and they are also very selective providing an efficient separation of both inorganic antimony species.

11.2.2 Complex formation with immobilized reagents

Several methods have been described for the determination of antimony based on the complexing ability of one of the antimony oxidation states, for instance, Sb(III) complexation with citrate immobilized on Dowex 1-X8 resin [34]. Antimony has been determined by HPLC on a reverse phase column modified with cyanide or diol groups retaining the complexes of Sb(III) with ethylxanthates [35], with hydroxamic acid [36], or with dithiocarbamate [37] in the determination of antibilharzial antimonials. Sb(III)-dithiocarbamate complexes have also been used in the determination of antimony by thin layer chromatography [38] and gas chromatography [39]. However, none of these methods has been applied to Sb(III) and Sb(V) speciation.

Chromatographic methods have not often been used for the selective determination of Sb(III) and Sb(V) as for the speciation of antimony compounds, such as chlorinated antimonials, using a Lichrosorb Si 60 column loaded with methyltriethylammonium chloride [40].

An elegant approach uses an immobilized enzyme as a chemical reagent to selectively react with certain species of an analyte. This method has potentials for both speciation and preconcentration. However, very few papers have been published on the use of enzymes in metal analysis [41].

It is known that schistosomicide antimonials act by deactivating the enzyme fructose-6-phosphate kinase by reacting with the thiol groups. Considering that schistomicides antimonials only produce their therapeutic effect in the trivalent state of antimony, and that Sb(III) is more toxic than Sb(V), De la Calle *et al.* [42] proposed the selective retention of Sb(III) with this enzyme. Using this method, Sb(V) and Sb(III) speciation was performed by the selective retention of Sb(III) with the enzyme fructose-6-phosphate kinase immobilized on controlled-pore glass.

The enzyme was immobilized on controlled pore glass using the method described by De León and Townshend [43]. Optimum conditions for Sb(III) retention by the immobilized enzyme were achieved by running the sample through the column at a flow rate of 0.2 ml.min⁻¹ and eluting the retained antimony with 1 % (v/v) lactic acid. The recovery of Sb(III) for spiked bidest and tap water under these conditions was 75%, and there was no need to control pH, temperature or ionic strength; recovery decreased to 65% for sea water.

The enzymatic method has, however, some disadvantages. The enzyme is not totally selective for Sb(III), since 25% of Sb(V) in deionized water and less than 10% of the Sb(V) in tap and sea water is retained on the column. Since the percentage of Sb(III) and Sb(V) retained by the enzyme depends on the matrix, the standard addition method must be used if the behaviour of the matrix to be analyzed is not previously known.

The volume of sample run through the column is a critical parameter, and antimony retention decreases drastically for volumes above 5 ml of deionized water and 1 ml of sea or tap water. This might be due to slight leaching by the samples, which slowly elute the retained antimony. This effect is more pronounced for sea and tap water because other concomitants in the matrix compete with antimony for the active sites of the enzyme.

Further experimental work is required to find a more selective enzyme for one of the antimony oxidation states and to improve preconcentration factors.

Apart from the immobilization of antimony complexes using columns, Sb(III) and Sb(V) speciation was also achieved retaining Sb(III) by formation of a complex with mercaptoacetic acid immobilized on cotton [44].

The sorption of ion exchangers on a polymeric support with catechol groups was the basis of a method of Khmylev *et al.* [45] for the preferential retention of Sb(V) over Sb(III); the inorganic exchangers: -SnO₂ and TiO₂ favoured the retention of Sb(III) over Sb(V).

11.2.3 Selective generation of antimony hydride

11.2.3.1 By pH control

This is, so far, the most common method for Sb(III) and Sb(V) speciation, together with the liquid-liquid extraction and determination by ETAAS.

Sb(III) is selectively reduced to stibine in the presence of Sb(V) at pH ≥ 2 (citric acid medium) [46,47], at pH ≥ 4 (tartaric acid medium) [46], in the pH 6-7 range [6], or even up to pH 8 (borate buffer) [48]. Campbell and Howard [49] succeeded to suppress the Sb(V) signal by generating the hydride at pH 5 (acetate buffer), and de la Calle *et al.* [50] quantitatively suppressed the Sb(V) signal by adjusting the pH 1.5-2 in H₃PO₄ (Fig.9). In this pH range the sensitivity of Sb(III) determination is the same as that achieved by generating the hydride in a strong acid medium such as 4 mol.l⁻¹ HCl; the signal is reduced by 10% at pH 2.5.

No conclusive explanation has been given so far to clearly understand this pH-dependent selective reduction. Agget and Aspell [51] suggested that it is due to the different reduction rate of Sb(V) by NaBH₄. Andreae *et al.* [6] proposed that the efficiency of the hydride generation process is strongly dependent on the pH of the reaction: the reduction yield normally decreases at pH values higher than pK_a, [pK_a(Sb(III))= 10 and pK_a(Sb(V))= 2.7)]. However, this did not explain the results

obtained by de la Calle *et al.* [50]. In order to determine whether antimony speciation is most influenced by the pH or by the particular medium used to obtain the pH, the authors prepared inorganic acid solutions at different pHs by adding appropriate amounts of NaOH to HCl, H₂SO₄, and HNO₃ solutions. The Sb(III) absorbance signal in HCl and H₂SO₄ remained constant at low pH and decreased at pH higher than 1, becoming negligible for pH > 2; in H₃PO₄ a pH higher than 3 was necessary to eliminate the Sb(III) signal. The Sb(V) absorbance signal drastically decreased between pH 0 and 1, becoming negligible for pH > 1. These results suggested that the efficiency of hydride generation depends not only on the pH but also on the medium used to generate the stibine, Fig.10.

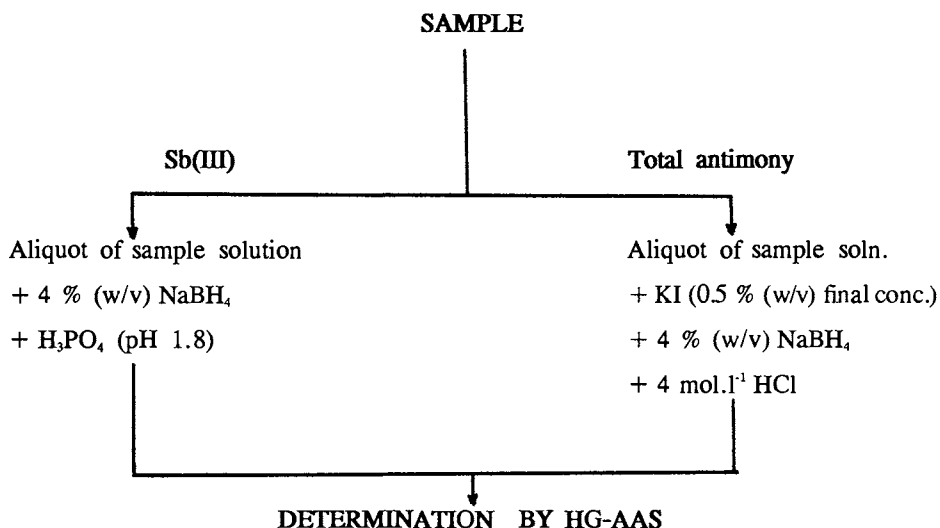


Figure 9: Sb(III) and Sb(V) speciation by HG-AAS under pH controlled conditions. Method proposed by De la Calle *et al.* [50].

With regard to the determination of total antimony, although Foreback [52], reported the complete reduction of Sb(III) in unbuffered solutions and of Sb(V) at pH 1.5-2.0 in the absence of reducing agent, most authors report a diminution in the Sb(V) absorbance signal with respect to that of Sb(III) [6,53]. Normally KI is added to the sample to reduce Sb(V) to Sb(III) and the stibine is generated in strongly acid medium of about 4 mol.l⁻¹ HCl, which provides good reproducibility and higher sensitivity and specificity than buffered media, Fig.9. The Sb(V) concentration is determined as the difference between total antimony and Sb(III), with all the drawbacks that this method involves.

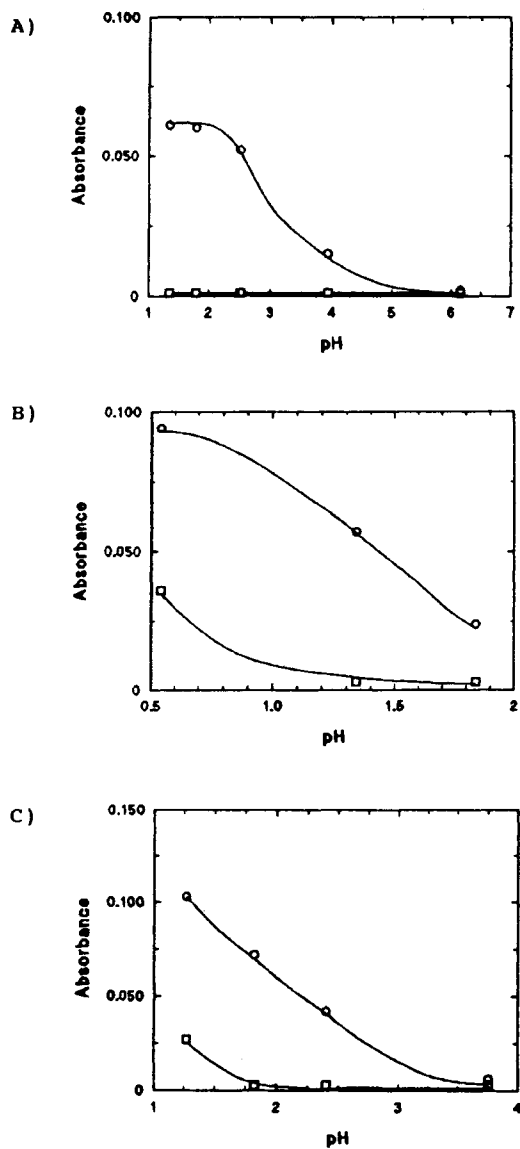


Figure 10: Comparison of the effect of pH on stibine generation with Sb(III)(80 ng.l⁻¹) and Sb(V)(200 ng.l⁻¹) using three different inorganic acids: A) H_3PO_4 , B) HCl , and C) H_2SO_4 . Sb(III); Sb(V).

The low detection limits normally required for antimony analysis in natural samples are mostly achieved by cold-trapping in U-tubes packed with glass beads [47] or glass wool [6] and immersed in a cold-nitrogen trap, which is removed after collection and preconcentration of the generated hydrides. Release of the collected stibine is at room temperature. A column packed with 15 % OV-3 on Chromosorb W 60/80 mesh is used by Andreae *et al.* [6], not only as a preconcentration system for Sb(V) and Sb(III) but also as a method for separating monomethylstibonic acid and dimethylstibinic acid. These methylated antimonials, which have been detected in natural samples, are best reduced in mildly acidic solutions (0.5 ml of 6 mol.l⁻¹ HCl in 100 ml of solution). Under these conditions, the absorbances of the methylated compounds are comparable to those of inorganic antimony. If the reduction takes place under the conditions used for Sb(III) at near-neutral pH, the monomethylstibonic acid peak decreases by about 30 % while that of dimethylstibinic acid does not change significantly. The two species are separated by removing the liquid nitrogen and heating the column with a wire wound around the outside of the trap column. To the best of our knowledge, this is the only work so far describing the speciation of methylated antimony compounds, Fig.11.

Sb(III) determination

- 1°) 100 ml of sample + 1 ml of Tris-HCl (1.9 mol.l⁻¹)(near neutral pH)
- 2°) Attach the reaction vessel to the apparatus and purge with helium for 3 min.
- 3°) Immerse the trap/column (15% OV-3 on Chromosorb W AWD MCS 60/80 mesh) in liquid nitrogen
- 4°) Inject 1 ml of 4 % (w/v) NaBH₄ (let stand 6 min. of reaction and stripping time)
- 5°) Remove the nitrogen and switch the variac (ca. 13V, 4A).

Total inorganic antimony determination

- 1°) 100 ml of sample + 2 ml of 9 mol.l⁻¹ HCl + 3 ml of 1 mol.l⁻¹ KI
- 2°) and 3°) As in Sb(III) determination
- 4°) Inject 3 ml of 4 % (w/v) NaBH₄
(let stand 6 min. of reaction and stripping time)
- 5°) As in Sb(III) determination.

Methylated antimonial compounds

- 1°) 100 ml of sample + 0.5 ml of 6 mol.l⁻¹ HCl
- The rest of the steps are common to those of Sb(III) determinations.

Figure 11: Sb(III), Sb(V), monomethylstibonic acid, and dimethylstibinic acid speciation procedure by cold trapping-HG-AAS, proposed by Andreae *et al.* [6].

Although AAS is the detection method most widely applied in antimony analysis by hydride generation, Sb(III) and Sb(V) have also been selectively determined by hydride generation at controlled pH using ICP-AES as the atomization and detection system. Nakahara and Kukui [54] determined total antimony in solution as follows. The sample is adjusted to 1 mol.l⁻¹ HCl, the Sb(V) is reduced to Sb(III) with KI or thiourea, and the hydride is generated with 1.5 % NaBH₄. In 1 mol.l⁻¹ malic acid or 0.5 mol.l⁻¹ tartaric acid, Sb(III) is selectively reduced to stibine by NaBH₄, thus allowing the differentiation of Sb(III) and Sb(V).

In general, the speciation of Sb(III) and Sb(V) by pH control has the disadvantage of determining only one of the species, Sb(III), at a near neutral pH, at which selectivity and reproducibility are poor. Furthermore, the predominant species, Sb(V), is always determined as the difference between total antimony and Sb(III), and therefore the accuracy is poor.

The hydride has traditionally been generated by batch systems, which have the disadvantages of high irreproducibility, time consuming analysis and high reagent expense. Other generation systems such as those based on a FIA design have been used more recently. De la Calle *et al.* [55] compared the speciation efficiencies of the FIA and continuous flow sample systems, for selective generation of stibine from Sb(III) using citric acid. Total antimony was determined by the addition of KI reducing agent and hydride generation in 4 mol.l⁻¹ HCl. Interference by Sb(V) is higher in the continuous flow system than in the FIA system. In the former, the interference of Sb(V) was about 5 %, while in the FIA system, no quantitative signal from Sb(V) was obtained.

11.2.3.2 Other methods

Mohammad *et al.* [11] observed that, with citric acid and acetic acid, speciation was not related to a pH effect (which was a secondary factor), but to the formation of a complex with Sb(V). The chemistry of the reaction of Sb(V) with citric acid and acetic acid has not yet been investigated; the observations, however, agree with the fact that antimony salts form complexes with certain acids, antimony being the nucleus of an anion [56]. The authors observed that the efficiency of hydride generation from Sb(III) increased with decreasing pH, so that a lower citric acid concentration was needed to achieve maximum efficiency, probably because of faster borohydride reduction at low pH. In contrast, the concentration of citrate required to suppress the Sb(V) signal decreases with decreasing pH, which suggests stable complexation of Sb(V) at low pH.

The list of complexant reagents that inhibit the generation of stibine from Sb(V) was extended by De la Calle *et al.* [10] to α -hydroxyacids other than citric acid, namely lactic and malic acid, although they also reported that the most effective inhibitor is citric acid, Fig.12.

This method of speciation allows the generation of stibine in a strongly acid medium such as 4 mol.l⁻¹ HCl, which provides higher reproducibility, sensitivity, and selectivity.

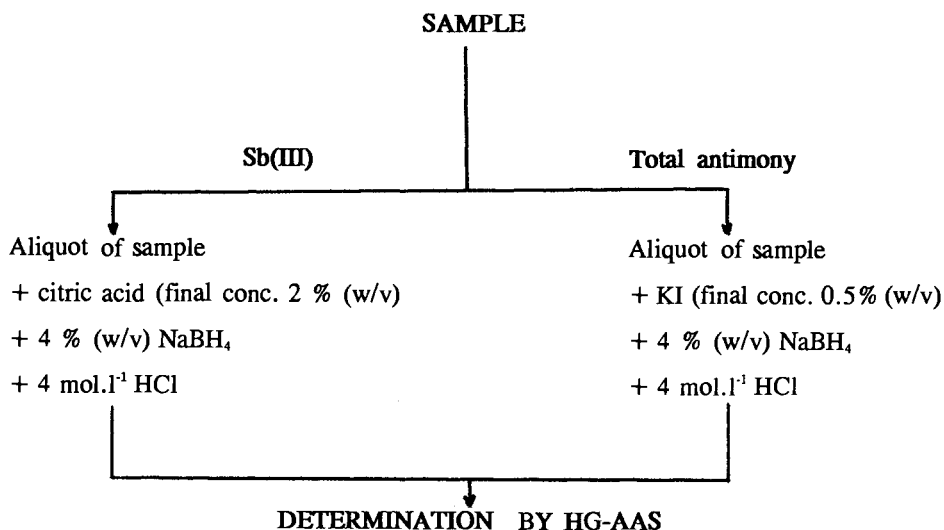


Figure 12: Sb(III) and Sb(V) speciation by complexation and HG-AAS, proposed by De la Calle *et al.* [10].

11.2.4 Electroanalytical methods

Although many electroanalytical methods of antimony determination based on the electroactivity of one of the antimony species have been described, few of them have been applied to the selective determination of antimony species. Apart from the work by Metzger and Braun [32] mentioned above, several methods have been described for the selective determination of Sb(III) and Sb(V) based on their different electroactivity under certain conditions.

Huang *et al.* [57] determined total antimony by electrolysis in a system containing a gold-fibre working electrode, an Ag-AgCl reference electrode and a platinum counter electrode in 20 $\mu\text{mol.l}^{-1}$ KI-4 mol.l^{-1} HCl medium, then stripping was effected at a constant current of 0.5 μA . They used exactly the same procedure in 20 $\mu\text{mol.l}^{-1}$ KI-0.1 mol.l^{-1} HCl medium to selectively determine Sb(III).

Gillain and Brihaye [58] combined differential pulse polarography at a hanging-mercury drop electrode and anode stripping voltammetry at a rotating ring-disc electrode to selectively determine Sb(III). Sb(V) is not electroactive and must be reduced to Sb(III) with gaseous SO₂ to determine the total concentration of antimony. Sb(V) is then determined as the difference between total antimony and Sb(III).

11.3 Conclusion

To summarize, nearly all the published papers dealing with inorganic antimony speciation are based on the determination of Sb(III), with Sb(V) being determined as the difference between total antimony and Sb(III). Thus poor precision and accuracy are usual in the determination of Sb(V).

Further efforts are necessary to improve the state of the art of antimony speciation analysis. Among them the validation of existing methods and the development of new analytical methods is particularly needed. Improvements will be made possible with the preparation of reference materials (RMs) to be used in interlaboratory studies and RMs certified for their contents in antimony species which are not available to date.

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12.

Arsenic speciation in environmental matrices

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Arsenic is widely distributed in the environment because of its natural origin and its industrial production. Arsenic compounds are mainly used in agriculture to prepare insecticides, herbicides and fungicides. They are also used as cotton dessicants or wood preservatives and in medicine as bactericides or parasiticides. The natural presence of arsenic is essentially due to the emergence of groundwaters containing high concentrations of that element and to volcanism. Terrestrial crust contains about 3 mg.kg⁻¹ As. In sea water as well as in freshwater, arsenic is present at the µg.kg⁻¹ level. In soils, contents are in the range of 0.05 to 0.2 mg.kg⁻¹. Marine organisms contain very high arsenic moieties (1-100 mg.kg⁻¹).

Arsenic occurs in various organic and inorganic species with several oxidation states (-3, 0, +3 and +5). The compounds most commonly found are arsenite and arsenate ions (As(III) and As(V)), monomethylarsonic and dimethylarsinic acids (MMA and DMA), arsine, di- and trimethylarsine as well as other organoarsenical compounds such as arsenobetaine (Asbet), arsenocholine (Aschol), arsenolipids and arsenosugars. Corresponding formula are presented in Table 1.

The toxicity of arsenic depends on its chemical form. Contrary to lead or mercury, inorganic species of arsenic are more toxic than organic compounds as shown in Table 2. It can be seen that the toxicity of As(III) and As(V) is to be compared to that of strychnine, which is known to be a violent poison. On the contrary, DMA and MMA are as toxic as aspirin. Asbet and Aschol are roughly not toxic.

Table 1: Chemical formula of some arsenic compounds

$\text{O} = \text{As} - \text{OH}$ <p>Arsenious acid (As(III))</p>	$\begin{array}{c} \text{OH} \\ \\ \text{O} = \text{As} - \text{OH} \\ \\ \text{OH} \end{array}$ <p>Arsenic acid (As(V))</p>	$\begin{array}{c} \text{CH}_3 \\ \\ \text{O} = \text{As} - \text{OH} \\ \\ \text{OH} \end{array}$ <p>Monomethylarsonic acid (MMA)</p>
$\begin{array}{c} \text{CH}_3 \\ \\ \text{O} = \text{As} - \text{CH}_3 \\ \\ \text{OH} \end{array}$ <p>Dimethylarsinic acid (DMA)</p>	$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3 - \text{As}^+ - \text{CH}_2 - \text{COO}^- \\ \\ \text{CH}_3 \end{array}$ <p>Arsenobetaine</p>	$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3 - \text{As}^+ - \text{CH}_2 - \text{CH}_2 - \text{OH}, \text{X}^- \\ \\ \text{CH}_3 \end{array}$ <p>Arsenocholine</p>
$\begin{array}{c} \text{OH} \\ \\ \text{O} = \text{As} - \text{C}_6\text{H}_5 \\ \\ \text{OH} \end{array}$ <p>Phenylarsonic acid</p>		

Table 2: Lethal Dose 50 of some arsenic compounds (LD₅₀: dose which is fatal to one-half a population of experimental animals)

Compounds	LD ₅₀ (mg.kg ⁻¹ weight of rat)
Arsine	3
Potassium arsenite	14
Arsenic trioxide	20
Calcium arsenate	20
Phenylarsonic acid	50
Monomethylarsonic acid (MMA)	700 - 1800
Dimethylarsinic acid (DMA)	700 - 2600
Strychnine	16
Aspirin	1000 - 1600
Arsenobetaine (Asbet)	> 10000
Arsenocholine (Aschol)	> 10000

In the environment, arsenic and its compounds may be submitted to physical, chemical and biochemical transformations with or without modification of their oxidation states, and/or mineralization, adsorption and precipitation processes. Figure 1 describes all the complex phenomena which may occur in the different compartments.

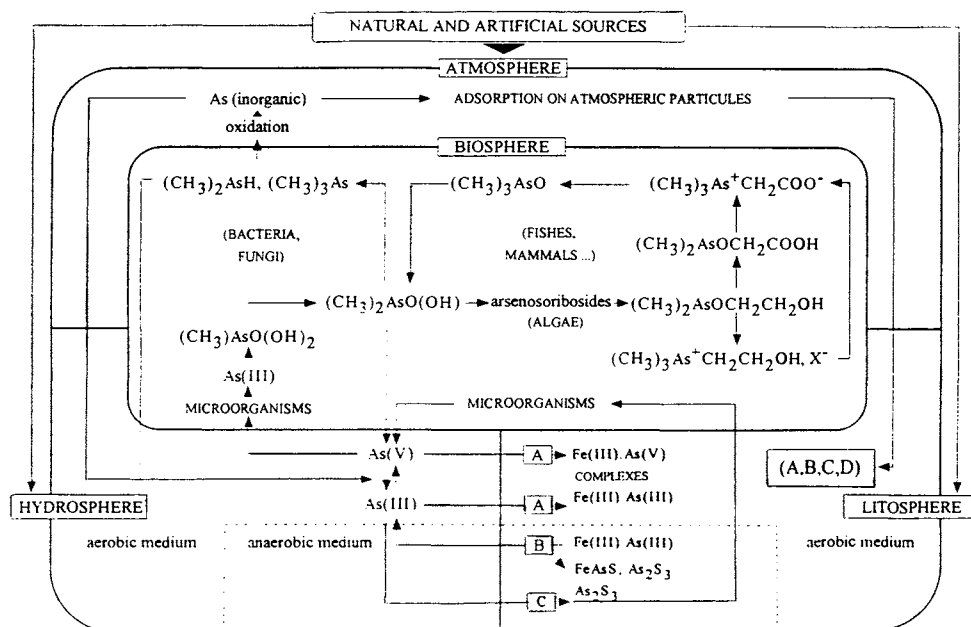


Figure 1: Bio-geochemical cycle of arsenic
A: deposition and co-precipitation, B: dissolution, C: diffusion and reaction with sulfides, D: adsorption

Speciation of arsenic has already been studied but has generally been performed on calibrant solutions. A Measurements and Testing Programme (BCR/EC) project concerning arsenic speciation in marine organisms is in progress. The aim is to prepare certified materials (mussels and tuna fish tissues) for six arsenic species: As(III), As(V), MMA, DMA, Asbet and Aschol. Different interlaboratory studies have been conducted and have shown the necessity of validating an analytical methodology.

12.1 Critical review of existing methods

The first methodology developed for arsenic speciation was based on the hydride generation technique [1]. Since then, other methods have been developed and detection limits have considerably decreased. Nowadays, it is possible to analyse samples containing only some tens ng.g^{-1} As of each individual species. Most of the techniques described in the literature are coupling methods using the hydride generation technique or liquid chromatography (LC) as separation methods and atomic absorption spectrometry (AAS), or inductively coupled plasma emission spectroscopy (ICP/AES) as detection technique. Direct current plasma emission spectroscopy (DCP/AES), or mass spectrometry have also been used. In this part, we present the most commonly used techniques, their advantages and their drawbacks.

12.1.1 Hydride generation method

12.1.1.1 Hydride generation and separation

This derivatization method was originally developed for selenium speciation [2] but has been successfully applied to the determination of arsenite and arsenate ions, monomethylarsonic and dimethylarsinic acids and trimethylarsenoxide.

In an acidic medium, some arsenic compounds may be reduced to volatile arsines and carried by an inert gas flow to a specific detector. Using suitable conditions, it is possible to generate arsines quantitatively and/or selectively. Main parameters are pH, as well as the nature and concentration of the reducing agent and the acid. In order to avoid problems due to the hydride generation kinetics, a liquid nitrogen trap is placed after the reactor. After reduction of the arsenic compounds, hydrides are carried to the trap where they are condensed. After complete reaction, the trap is progressively heated and hydrides are volatilized according to their respective boiling points. This technique, also used for tin speciation, is known as the "cold trap method". Arsines formed are further separated by a gas chromatograph or analysed directly.

Many authors have studied the selectivity of hydride generation as a function of reactional parameters in the case of arsenic [3-9]. Anderson and coworkers (1986) have shown that it was possible to selectively generate hydrides from As(III), As(V), MMA and DMA. Conditions described allow the rapid determination of As(III), DMA, As(III) + As(V) and total arsenic concentrations [8].

12.1.1.2 Detection

Whatever the detection technique used, the main advantages of the hydride generation technique are:

- analyte/matrix separation
- improvement of the introduction system: the quantity introduced to the detector is 50 to 100 higher than with pneumatic nebulizers
- analyte preconcentration
- large choice of detection techniques
- easy on-line and automatized use

This method has, nevertheless, several drawbacks:

- quantification is possible only for compounds forming volatile hydrides (arsenobetaine and arsenocholine, for example, can not be converted into arsines)
- the presence of some elements (Ni, Co, Cu, ...) decreases the efficiency of hydride generation
- one must strictly control the reaction medium to obtain good results.

The hydride generation technique was first coupled to flame atomic absorption spectrometry (FAAS) [10]. The analyte is removed from the matrix so that classical interferences observed in AAS are reduced or even eliminated. Nevertheless, FAAS is not sensitive enough to allow the determination of arsenic traces in environmental samples and flames have been progressively replaced by quartz furnaces [8,11] which offer better control of atomization and lead to more suitable detection limits. Using this technique, inorganic species (arsenite and arsenate), monomethylarsonic and dimethylarsinic acids have been quantified in waters, sediments and biological tissue extracts [3,8,9,12-14] with detection limits in the $\mu\text{g.l}^{-1}$ range.

Atomic emission spectrometry is not often used because of its higher cost. Hydride generation has been only coupled with a direct current plasma system for the determination of arsenite, arsenate and total arsenic in waters and fishes [15].

The most sophisticated system has been developed by Kaise *et al.* [16]. Hydrides are generated, condensed, volatilized, separated by gas chromatography and then analysed in a mass spectrometer. The most intense peaks correspond to $m/z = 76$ and 78 for AsH_3 , 90 for CH_3AsH_2 and $(\text{CH}_3)_2\text{AsH}$, 103 and 120 for $(\text{CH}_3)_3\text{As}$.

12.1.2 Liquid Chromatography coupled with specific detectors

Liquid chromatography is particularly suitable for the determination of arsenic compounds because of the hydrophilic and ionic or ionizable character of these species. This technique presents several advantages when compared to the previous one. Indeed, no derivatization is needed before separation, several types of chromatography may be used and a large choice of stationary phase/mobile phase couples are available. Nevertheless, classical detectors such as UV/Vis or electrochemical detectors are not sensitive enough and a specific detector (absorption or emission spectrometry) has to be used.

12.1.2.1 Separation by ion-pair chromatography

Separations have been performed using apolar stationary phases constituted of C18 silica [17-20] or polymeric resin [21-23]. The ion-pairing agent is generally tetrabutylammonium phosphate [17,18] or hydroxide [20-23]. Brickman *et al.* [24] have achieved the separation in the presence of tetraheptylammonium nitrate whereas Larsen has investigated C₈-C₁₂ alkylammoniums as well as butanesulfonate [19]. Elution is achieved using an isocratic regime or a gradient step program. Analysis time is in the range of 10 to 40 minutes.

12.1.2.2 Separation by ion-exchange chromatography

Silica based [18,20,25-27] or polymeric [19,29-35] stationary phases grafted with strong anion-exchange groups (quaternary ammoniums) as well as weak anion-exchange columns [28] have been investigated. Compounds are eluted using a phosphate, carbonate or acetate buffer as mobile phase. pH is in the 6-7 range. The addition of an organic modifier has been found to decrease hydrophobic interactions [23-25]. Analysis times are also in the range of 10 to 40 minutes. These separations have been performed on calibrant solutions and on natural samples: pesticides and herbicides residues [29], urine [28,30,36], biological samples [25,26,32] and waters [31]. Some authors have mentioned the degradation of silica ion-exchange columns after few weeks of extensive use [27,30,33].

12.1.2.3 Detection by atomic absorption spectrometry

Coupling HPLC with FAAS does not present any major technological problems. Nevertheless, the low sensitivity of the system (detection limit for arsenic : 1 mg.l⁻¹) due to the difficult introduction of the sample to the flame and to the attenuation of the arsenic radiation intensity by radicals formed in the flame, has considerably limited its application in the arsenic speciation field.

Several studies using electrothermal atomic absorption spectrometry (ETAAS) as the detector have shown that sensitivity can be improved by a factor of 10 to 100 when compared to FAAS. Nevertheless, coupling a continuous separation technique (HPLC) to a sequential detector is not easy. Two types of interface have been used, leading to off-line and on-line coupling methods:

- in the first [37], a graphite furnace sampler is used as a fraction collector. Better resolution is obtained but analysis time is considerably increased.
- in the on-line method [38-40], effluent fractions are collected and periodically analysed. This technique requires large chromatographic peaks because 30 to 60 s are needed for each determination.

HPLC-ETAAS has been developed for arsenic speciation using anion-exchange [38,39] and reverse phase liquid chromatography [24]. Mobile phases, generally more complex, lead to an important increase of the background noise and require the use of a powerful apparatus equipped with a Zeeman background correction system [41]. It has been used for arsenic speciation in waters [24], pesticides [29,39] and soils [24]. Detection limits are in the nanogramme range. However, elution of significant quantities of organic materials together with arsenic compounds disturbs the detection. Moreover, some species are able to be volatilized without atomization if large amounts of salts or carbonaceous compounds are present in the sample.

12.1.2.4 Detection by inductively coupled plasma atomic emission spectrometry

The compatibility of flow rates used in HPLC and ICP/AES (generally 1 ml.min⁻¹) allows a very easy coupling between these two techniques. A PTFE tube of adequate internal diameter is connected from the exit of the column to the nebulizer of the apparatus.

This coupling method has been developed for arsenic speciation with ion-exchange [18,26,32,42-44] and ion-pair liquid chromatography [17,21,45]. Detection limits are some tens of nanogrammes for ion-exchange chromatography or some hundreds of nanogrammes for ion-pair liquid chromatography. Some attempts to decrease the detection limits by improving the introduction system have been realized but no conclusive results have been obtained. The detection limits obtained allow the determination of the most abundant arsenic compound found in marine food, *i.e.* arsenobetaine, but are generally not low enough to reach the other concentrations.

12.1.2.5 LC procedures involving hydride generation

Matrix interferences observed in LC-AAS may be suppressed and detection limits improved by a post-column derivatization: for example the reduction of arsenic species into volatile arsine increases the quantity of analyte introduced in the atomization cell. Hydrides are carried by an inert gas current into a heated quartz cell where they are atomized and detected. Detection limits are in the range of few hundreds of picogrammes As, which are particularly suited for arsenic determination in environmental samples.

ICP-AES detection may also be used after hydride generation in a simple coupling configuration: a PTFE tube is connected from the column to the entrance of the reduction coil, the nebulizer of the ICP/AES is removed and the exit of the gas-liquid separator is directly connected to the plasma. This modification may be realized by the user himself but some hydride generation kits are commercially available.

The use of hydride generation for arsenic speciation increases sensitivity (some nanogrammes) but restricts the application of the technique to species which are able to form hydrides *i.e.* As(III), As(V), MMA and DMA. Some authors have proposed a photolytic reaction to convert Asbet, MMA and DMA in As(V) [46,47]. Nevertheless, the long period of time (hours) required for quantitative photolysis of Asbet makes it unsuitable for "on-line" measurements. The UV irradiation with addition of peroxodisulfate has been used for Asbet, DMA, MMA and As(V) determination in a LC-UV-HG-ICP system [48].

Recently, Rauret *et al.* have studied the photo-oxidation of Asbet and Aschol by UV irradiation in persulfate media in order to convert these inert compounds in simple molecules able to produce volatile hydrides which could be determined by LC-UV-HG-ICP/AES [49]. The experimental conditions such as the photoreactor design, use of persulfate in different media, irradiation time and the effect of the power lamp have been optimized and it was shown that all the arsenic species of environmental interest are able to be converted into As(V) and consequently to be quantified by the LC-UV-HG-ICP/AES technique. Detection limits are very close to those obtained with HG-ICP/AES detection.

12.1.2.6 HPLC coupled to ICP-MS

This coupling method has been used for the speciation of arsenic with ion exchange, ion-pair, or gel permeation chromatography [22,28,33,34,50-57].

Detection limits are very low (20-200 pg As) and the detector is linear over a wide dynamic range. This technique has been successfully applied to the determination of arsenic compounds in seafood products and sediments [50, 52].

12.2 Means of validation

Some reference materials certified for total arsenic concentration are commercially available. In the environmental field and more particularly concerning the biological tissues and sediments CRM's, the Measurements and Testing Programme (BCR) of the Commission of the European Communities provides 7 materials:

- CRM 185 (bovine liver) :	$24 \pm 3 \mu\text{g}\cdot\text{g}^{-1}$
- CRM 186 (pig kidney) :	$63 \pm 9 \mu\text{g}\cdot\text{g}^{-1}$
- CRM 278 (mussel tissue) :	$5.9 \pm 0.2 \mu\text{g}\cdot\text{g}^{-1}$
- CRM 422 (cod muscle) :	$21.1 \pm 0.5 \mu\text{g}\cdot\text{g}^{-1}$
- CRM 277 (estuarine sediment):	$47.3 \pm 1.6 \mu\text{g}\cdot\text{g}^{-1}$
- CRM 280 (lake sediment) :	$51.0 \pm 2.4 \mu\text{g}\cdot\text{g}^{-1}$
- CRM 320 (river sediment) :	$76.7 \pm 3.4 \mu\text{g}\cdot\text{g}^{-1}$

The National Research Council Canada (NRCC) has also prepared two fish tissues certified for their total arsenic content:

- DORM-1 (dogfish muscle) :	$17.7 \pm 2.1 \mu\text{g}\cdot\text{g}^{-1}$
- DOLT-1 (dogfish liver) :	$10.1 \pm 1.4 \mu\text{g}\cdot\text{g}^{-1}$

Finally, the National Institute of Standards and Technology (NIST) produced two sediments:

- SRM 1646 (estuarine sediment) :	$11.6 \pm 1.3 \mu\text{g}\cdot\text{g}^{-1}$
- SRM 2704 (buffalo river sediment) :	$23.4 \pm 0.8 \mu\text{g}\cdot\text{g}^{-1}$

Speciation studies have been conducted on DORM-1 CRM [50,54,57] and have shown that 91 to 96 % of the arsenic extracted in the aqueous phase is present as Asbet. Nevertheless, no reference material certified for arsenic species is available. A BCR project which aims to elaborate reference materials of this type is now in progress. Pilot laboratories are the Laboratoire de Chimie Minérale et Analytique (Strasbourg, F) and the Service Central d'Analyse (CNRS, Vernaison, F). At the beginning, four materials (soil, sediment, fish and mussel tissues) and six arsenic species (As(III), As(V), DMA, MMA, Asbet and Aschol) were proposed. Nevertheless, the idea of certifying soils and sediments was abandoned because of the difficulty of collecting the appropriate materials.

Moreover, the easy interconversion between As(III) and As(V) has led the organizers to restrict the quantification to As(III)+As(V), Asbet, Aschol, DMA and MMA.

Intercomparison studies involving 10 to 20 laboratories have been organized using the classical step by step approach:

- 1- Preparation of the calibrants
- 2- Study of pure calibrants or mixtures of pure calibrant solutions
- 3- Study of mixtures of calibrants containing interfering compounds
- 4- Study of clean extracts
- 5- Study of raw extracts
- 6- Study of real samples

Each step involves one or two intercomparison exercises and stability studies of the solutions distributed to the participants. With respect to calibrants, As(III), As(V), DMA and MMA are commercially available and can be used to prepare calibrant solutions. On the contrary, Asbet and Aschol have to be synthesized. The preparation has been achieved at the Laboratoire des Matériaux Organiques (Solaize, France) and characterized by elemental analysis, mass spectrometry and inductively coupled plasma atomic emission spectrometry.

Calibrant solutions have been prepared in freshly boiled deionized water and their stability studied at 4 °C, 25 °C and 40 °C. It has been proved that solutions are stable at least for six months if kept in the dark at 4 °C. If storage conditions are not respected, oxidation of As(III) into As(V) and degradation of methylated species may occur.

Stability studies performed for steps 3, 4 and 5 have shown that solutions which do not contain inorganic arsenic are stable at -40 °C and 4 °C in the dark. In the presence of mineral arsenic, interconversion between As(III) and As(V) is observed, this phenomenon depending on the temperature and on the sample composition.

The different intercomparison studies have helped the participants to improve their methods of determination and have allowed the identification of several sources of error (problem of calibration, column washing and pre-conditioning, sample treatment, presence of chlorides for ICP-MS detection *etc.*) which have been solved. The 6th step has been started in the second half of 1994 and the certification campaign of mussels and fish tissues has been organized at the end of 1994.

12.3 Description of a validated technique

Sample treatment and arsenic speciation procedures described in this paragraph are the methods used in laboratories 1 (Laboratoire de Chimie Minérale et Analytique, Strasbourg), 2 (Service Central d'Analyse, Vernaison) and 3 (Universitat de Barcelona, E).

These procedures have been established for the six more commonly found arsenic species in sediments and seafood products: As(III), As(V), Asbet, Aschol, DMA and MMA.

12.3.1 Sample treatment

The sample preparation is one of the most difficult steps for trace element speciation in natural samples. The entire procedure has to be achieved in such a way that no loss or contamination or change in the speciation happens.

12.3.1.1 Analysis of seafood materials

Fresh material is collected, washed with sea water and stored at 0°C. In the case of shellfish, shells are opened by freezing at -25 °C. After that, products are lyophilized so that the moisture content does not exceed 8-9 %. The powder obtained is homogenized, pulverized and filtered on a 125 µm sieve.

Further treatment applied by Lab.1 to this type of sample is based on a water/methanol extraction followed by a clean-up with ether as shown in Figure 2. Methanol/water evaporation should not be taken to dryness because of the possibility of volatilization and/or degradation. In the extraction step, the final volume indicated corresponds to the minimal volume required to take the sample out from the flask. It may be reduced if possible but practically can not be lower than 5 ml. Indeed, the solution becomes too viscous and further purification can not be achieved. Filtration may be replaced by centrifugation in the extraction step. Use of an ultrasonic bath has been preferred to mechanical stirring because sample powder is more easily dispersed that way.

In order to check that the procedure does not induce any transformation of the species of interest and leads to 100 % recovery, a codfish powder was:

- first spiked with calibrant solutions of the six species of interest and then extracted and purified (I)
- secondly extracted, purified and spiked (II).

Speciation was then performed using LC-ICP/AES (for Asbet and Aschol) and LC-HG-QFAAS coupling techniques. The same results were obtained by procedures I and II. Moreover, total arsenic concentration independently determined by EDXRF (Energy Dispersive X-Ray Fluorescence) on the starting powder was found equal to the sum of Asbet, DMA and As(V) concentrations, the only As species detected in the sample. Finally, concentrations found for each compound in procedures I and II corresponded to the sum: concentration in the sample + concentration of the spike.

12.3.1.2 Sediment analysis

In sediments, the most commonly found arsenic species are As(III), As(V), DMA and MMA. Extraction may be performed by classical acid attack in beaker or tubes or by microwave digestion after homogenization, pulverization and filtration on a 125 µm sieve. The advantages of microwave digestion are both the reduction of analysis times and the possibility of automatization.

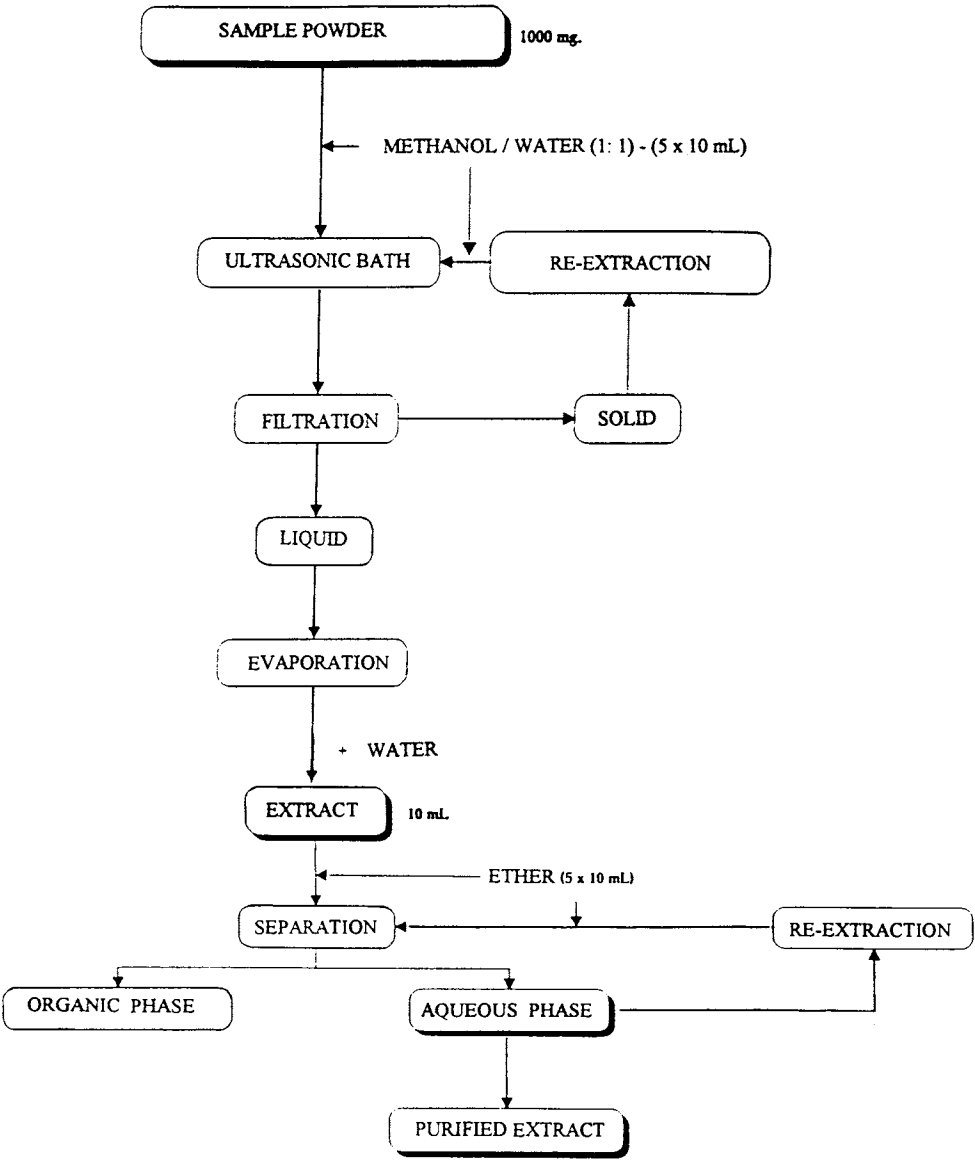


Figure 2: Extraction/Clean-up procedure for arsenic speciation in seafood products

Two procedures have been developed by laboratory 2 for sediment analysis [58]:

Digestion with a mixture of hydrochloric and nitric acids:

7 ml HCl and 3 ml HNO₃ are added to 200 mg powder sample and introduced into a microwave digestion apparatus (Microdigest A 300 type, Prolabo, power: 0-200 W) using 20 % of maximum power for 5 minutes and then 25 % maximal power for 10 minutes. The same quantity of HCl/HNO₃ mixture, 1 ml H₂O₂ and 5 ml water are successively added to the solution and heated at 30 % for 10 minutes, 20 % for 5 minutes and 25 % for 5 minutes respectively. It was observed by HPLC-ICPMS that, under those conditions, As(III) is totally converted into As(V) but DMA and MMA are preserved. This procedure allows the determination of mineral arsenic, DMA and MMA.

Digestion with orthophosphoric acid:

The best extraction yields are obtained using orthophosphoric acid 0.3 mol.l⁻¹ at pH 1.3 stirring the solution (100 mg powder and 15 ml acid) for 10 minutes in the same microwave system as previously described. In those conditions, As(III) oxidation is slight. DMA and MMA can quantified.

12.3.2 Arsenic speciation

The separation technique used by laboratories 1, 2 and 3 is liquid chromatography. Lab. 1 performed the separation using an ion-pair reverse system followed by ICP/AES and HG-QFAAS techniques whereas labs. 2 and 3 used ion-exchange chromatography with ICP-MS and UV-HG-ICP/AES detections respectively. Arsenic was measured at a wavelength of 193.7 nm.

12.3.2.1 Procedures used: Laboratory 1

- Determination of arsenobetaine by ICP/AES

The first approach is based on the direct connection between the chromatographic system and the atomic emission spectrophotometer. The interface between both is made of a PTFE tube (250 μm, length: as short as possible) which is connected from the exit of the column to the nebulizer of the apparatus. Taking into account the poor sensitivity of the technique (see paragraph 12.1), only arsenobetaine can be determined this way in seafood products. 200 μl of the purified extract is injected onto a Hamilton PRP-1 ion-pair column using a solution of tetrabutylammonium phosphate (TBAP, 0.5 mmol.l⁻¹, pH 9.5) as a mobile phase. The eluate is directly introduced into the ICP/AES nebulizer and analysed.

- Determination of As(III), As(V), DMA and MMA by LC-HG-QFAAS

Chromatographic conditions were modified to separate the four arsenic species forming hydrides in one run. This time, 200 μl of the sample was injected onto the same column but with a mixture of TBAP (10 mM) and TBAOH (Tetrabutylammoniumhydroxide, 10mmol.l⁻¹) at pH 6.15 as a mobile phase. The pH of the mobile phase as well as buffer concentration have been optimized. In the derivatization phase, the type of acid, its concentration and borohydride concentration have been considered. H₂SO₄ (0.5 mol.l⁻¹, 1 ml.min⁻¹) and NaBH₄

(1 % in NaOH 0.1 %, 1 ml.min⁻¹) were added to the eluate and the solution was introduced into the gas-liquid separator of the hydride generation system (Perkin Elmer FIAS 400, shown in Figure 3a). The separator includes a membrane which prevents the liquid entering the quartz atomization cell. Nevertheless, the mobile phase used becomes very effervescent when acid and borohydride are added and membrane is often wet if no precaution is taken; one solution consists of inserting a spring into the separator. The argon flow rate was set at a value of about 75 ml.min⁻¹ which leads to the best compromise between reproducibility and sensitivity. Cell temperature was about 900 °C and the slit width was set at 0.7 nm. An EDL lamp (20 mA) was used.

12.3.2.2 Procedures used: Laboratory 2

- Determination of As(III), As(V), DMA and MMA by LC-HG-QFAAS

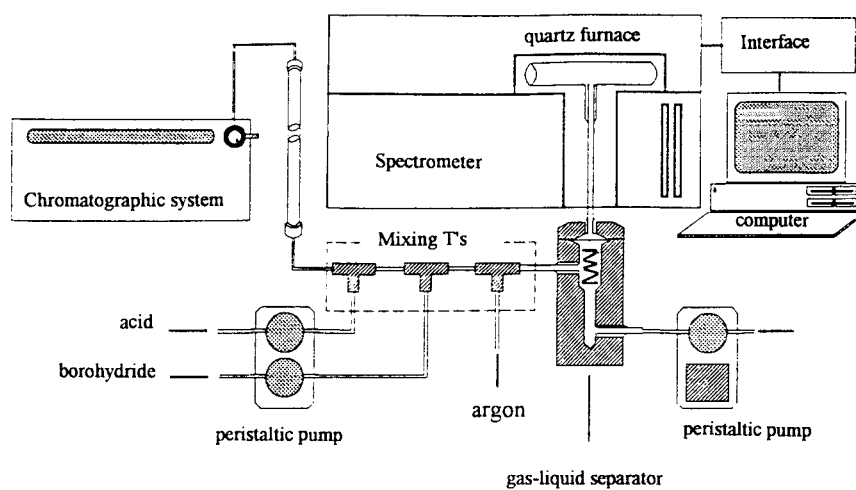
The same method as Lab. 1 has been used. Only the LC conditions and hydride generation system (FIAS 200, Perkin Elmer) were different.

100 µl of sample solution was injected onto a Hamilton PRP X-100 anion exchange column and a gradient elution was performed using a flow rate of 1 ml.min⁻¹. As mobile phases solution A ((NH₄)₂PO₄/(NH₄)₂HPO₄, 10 mol.l⁻¹, pH 6.2) and solution B ((NH₄)₂HPO₄, 100 mol.l⁻¹, pH 8) were used. The following gradient program was performed : 100 % A for 3.4 min, decreasing to 50 % A in 0.1 min and maintained for 3 min. 100 % A was reached again in 0.1 min and maintained for 7 min. This mobile phase did not become effervescent when acid and reductant were added. H₂SO₄ 1 mol.l⁻¹ (1.3 ml.min⁻¹) and NaBH₄ (1 % in NaOH 0.1 %, 1.3 ml.min⁻¹) were used as reactants for hydride generation. Reaction takes place in a PTFE tube (25 cm length, 0.5 mm i.d.). Detection limits were similar to those obtained by Lab.1.

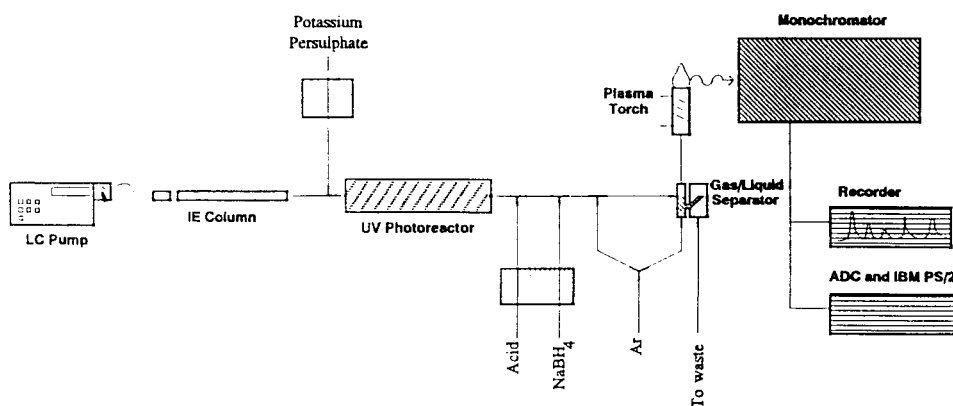
- Determination of all the species by HPLC-ICP-MS [51]

LC conditions were adapted to the specific detector used. Separation was performed using the mobile phases described previously but adding 2 % CH₃CN to enhance the sensitivity of the method. NH₄⁺ counter ions are preferred to Na⁺ ions because signals observed in ICP-MS are greatly affected by the presence of alkaline ions. Parameters of the ICP-MS apparatus (VG Plasma Quad 2) were optimized each day with a solution of 20 ng.ml⁻¹ As in buffer A in order to reach the highest possible signal.

Coupling between LC and ICPMS system was achieved with a simple PTFE connection as previously described. As was determined at m/z 75. The signal was recorded on a Shimadzu CR3A integrator connected to the analog output of the electron multiplier (pulse counting mode). A resistance-capacity filter was used in order to lower the background noise. High concentrations of easily ionizable cations such as Na⁺ or K⁺ could interfere with As determination because of their similar retention times. Chloride ions, which may combine with argon to produce an interfering peak at m/z 75 in ICP-MS, cannot disturb arsenic speciation in this system since they elute later than the arsenic compounds of interest.



A



B

Figure 3: Schematic of the (A) LC-HG-QFAAS and (B) LC-UV-HG-ICP/AES systems used for arsenic speciation

12.3.2.3 Procedures used: Laboratory 3

- Determination by HPLC-UV-HG-ICP/AES

As already said, ICP/AES allows the detection of Asbet if its concentration is high enough (for example, in seafood products) and HG-ICP/AES is limited to the quantification of species which are able to form hydrides. In order to determine all the species on line and in one experiment, Lab.3 has developed a method in which they are all converted into As(V) in a photo-reactor located just after the LC chromatograph. The As(V) obtained is introduced into an hydride generation system and determined by ICP/AES.

Working conditions have been optimized in order to obtain the best detection limits. The signal is filtered by using a Fourier Transform in order to better evaluate the peak height.

A photoreactor was developed to be easily coupled on line between the exit of the HPLC column and the entrance to the reduction chamber. The length and the internal diameter of the PTFE coil and flow rate, were considered in order to minimize the dispersion of the chromatographic peaks, since this depends on L , r and, according to the equation:

$$\sigma_V^2 = k \frac{\pi r^4 L}{24 D_m} \phi$$

r : internal radius of the capillary

ϕ : liquid flow rate

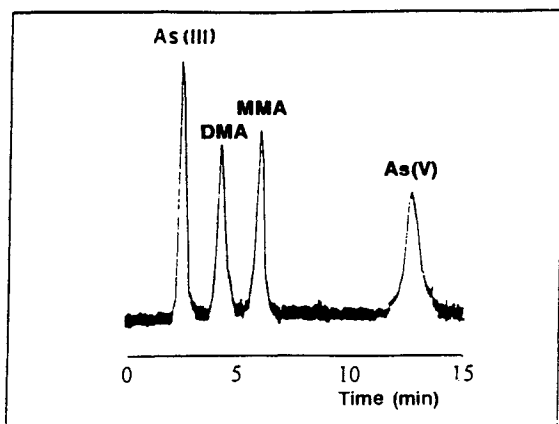
D_m : molecular diffusion coefficient for analyte in solvent

k : proportionality factor which depends on the flow profile in the capillary

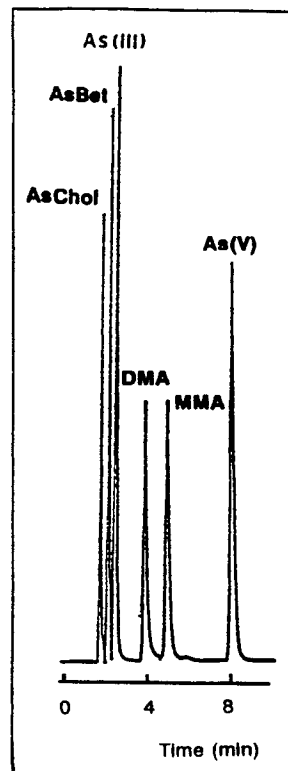
The variance can be minimized when the length of the tube is increased and its internal diameter is reduced. Thus, 0.35 mm, the narrowest easily available PTFE tube was chosen. The optimal length of the tube was determined by the time necessary to complete the photo-oxidation reaction. This photo-oxidation permits "on line" coupling between chromatographic separation and hydride generation.

The eluate is introduced in the photo-reactor connected to the outlet of the column with addition of 3 % $K_2S_2O_8$ in 3 % NaOH (0.2 ml.min^{-1}) as photo-oxidation reagent. The vapour emerging from the photo-reactor is introduced into the hydride generation system. As hydride generation system, HCl 8 mol.l^{-1} (1 ml.min^{-1}) and NaBH₄ 1 % in NaOH 0.5 % (1 ml.min^{-1}) are used. The resulting solution reaches the gas-liquid separator and then the plasma torch (see Figure 3b).

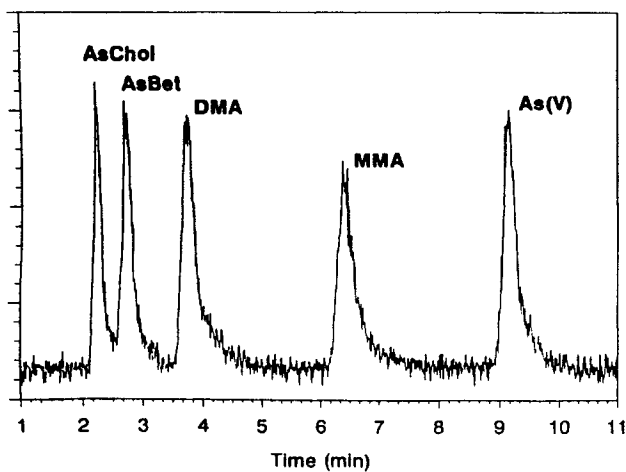
Figure 4 presents some chromatograms obtained by the arsenic speciation methods developed in the three laboratories. Detection limits and reproducibility of the techniques are shown in Tables 3 and 4.



A



B



C

Figure 4: Chromatograms obtained by Labs. 1, 2 and 3 using (A) LC-HG-QFAAS, (B) LC-ICPMS and (C) LC-UV-HG-ICP/AES techniques (chromatographic conditions described in the text)

Table 3: Detection limits (in ng As) of the four detection techniques considered in this study (ICP/AES, UV-HG-ICP/AES, HG-QFAAS and ICPMS)

Compound	LC-ICP/AES (Lab.1)	LC-UV-HG- ICP/AES (Lab.3)	LC-HG QFAAS (Lab.1)	LC-ICPMS (Lab.2)
As(III)	120	0.26	0.15	0.01
As(V)	130	0.96	0.84	0.03
MMA	100	1.3	0.33	0.02
DMA	130	0.98	0.43	0.02
Asbet	110	0.79	----	0.01
Aschol	120	0.61	----	0.01

Table 4: Coefficients of variation expressed in % of the four detection techniques considered

Compound	LC-ICP/AES (Lab.1) ^a	LC-UV-HG- ICP/AES (Lab.3) ^b	LC-HG QFAAS (Lab.1) ^c	LC-ICPMS (Lab.2) ^d
As(III)	2.42	5.5	3.12	2.2
As(V)	3.90	5.2	3.67	1.6
MMA	4.71	6.3	3.46	4.1
DMA	2.76	6.8	2.95	2.5
Asbet	3.55	5.0	----	2.5
Aschol	2.37	4.6	----	2.4

^a short term reproducibility considered for eight consecutive injections of a solution 10 $\mu\text{g}.\text{ml}^{-1}$ (in As).

^b long term reproducibility determined by injecting ten times in three non consecutive days a solution approximatively five times the LOD.

^c short term reproducibility determined by injecting eight consecutive times a solution 50 $\text{ng}.\text{ml}^{-1}$ (in As) of each compound .

^d short term reproducibility determined by injecting six consecutive times a solution 0.1 $\text{mg}.\text{ml}^{-1}$ of each compound .

12.4 Conclusions

Several coupling techniques using liquid chromatography as the separation method and ICP/AES, HG-QFAAS, UV-HG-ICP/AES or ICP-MS as detectors are now available for arsenic speciation in environmental matrices. Nevertheless, some inter-comparisons within the BCR programme on arsenic speciation in marine organisms and sediments have shown discrepancies between the results of the different laboratories involved. Further efforts are

necessary to improve the quality of the measurements, particularly at sample preparation step as well as on the quantification procedure.

Concerning the development of new methods for arsenic speciation, capillary zone electrophoresis (CZE) appears *a priori* to be suitable since it is best dedicated to ion separation. As(III), As(V), DMA and MMA have already been separated at low concentrations using CZE [59] and the technique has been evaluated in a BCR intercomparison study (Figure 5). However, when applied to extracts of environmental samples the detection limits (UV detector, 190 nm) are too high for the arsenic species. CZE technique can be coupled with mass spectrometry but the low masses of the compounds do not allow good detection limits.

Improvements are also needed in the clean-up procedure of the extracts. Additionally the extraction leads to a significant dilution and therefore requires very low detection limits (5 to 10 ng.ml⁻¹) which are best fitted by ICP-MS or HG-GFAAS. Another development would be the obtention of specific extractants designed for anion extraction. This would possibly lead to pre-concentration using either liquid-liquid extraction or substituted resins.

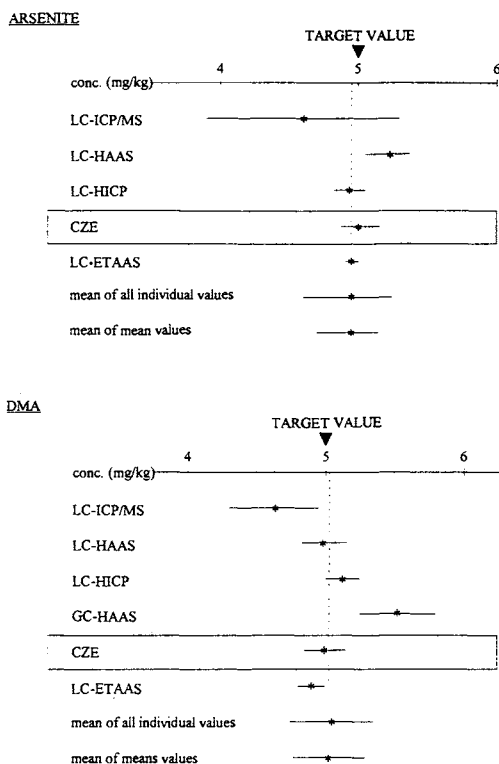


Figure 5: Results of a BCR intercomparison study for As speciation in a mixture of standard solutions containing 5 mg.kg⁻¹ of the different species. Example of As(III) and DMA.

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13.

Mercury speciation in biological matrices

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In nature, mercury occurs in several forms, *e.g.* metallic mercury, inorganic mercury and organic mercury compounds. All forms of mercury are considered poisonous, but methyl-mercury is of particular concern since it is extremely toxic and is frequently found in the environment. Through a very effective biomagnification mechanism, methyl-mercury is enriched in food chains which results in high levels in top predators, *e.g.* fish such as northern pike and tuna. In Minamata (Japan), methyl-mercury contamination caused severe brain damage of 22 infants whose mothers had ingested contaminated fish during pregnancy [1]. Intake of wheat flour from seeds treated with organic mercury has also led to large scale poisoning, *e.g.* methyl-mercury treated seeds caused a dramatic contamination of humans in Iraq in 1971-72 [2]. In general, exposure to organic mercury can cause brain damage to a developing foetus [3]. The exposure is considered to be more dangerous for young children because their nervous systems are still developing and thus are more sensitive to these compounds. The toxicity of mercury is extensively documented by WHO [4,5].

Due to (i) the toxicity of organic mercury, (ii) the fact that organo-mercury compounds can be formed in nature (methyl-mercury and probably dimethyl-mercury), and (iii) the bio-accumulation of methyl-mercury, there has been a great interest in trying to understand the distribution patterns in nature and the interconversion between the different mercury compounds. Many of these studies have been reported in various books and reviews, *e.g.* [6-9].

A study of the speciation of mercury is necessary to understand its pathway, to assess its toxicity and to develop strategies for its decontamination. As an example, the perception of the mercury cycle has undergone a tremendous change after the development of more accurate and sensitive methods for mercury speciation (Figs. 1a and 1b). It is now realized that instead of having one mercury cycle in which mercury essentially originates from the bio-methylation of mercury in sediments, methyl-mercury in fish can originate from an atmospheric depositional flux of methyl-mercury.

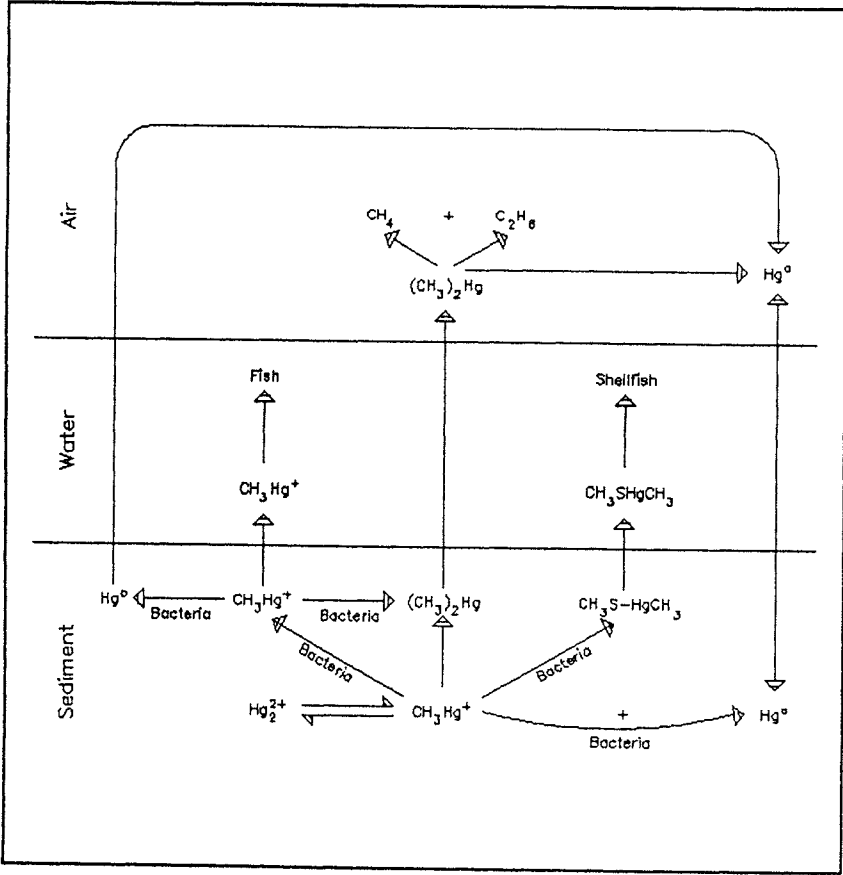


Figure 1a: Old perception of the mercury cycle

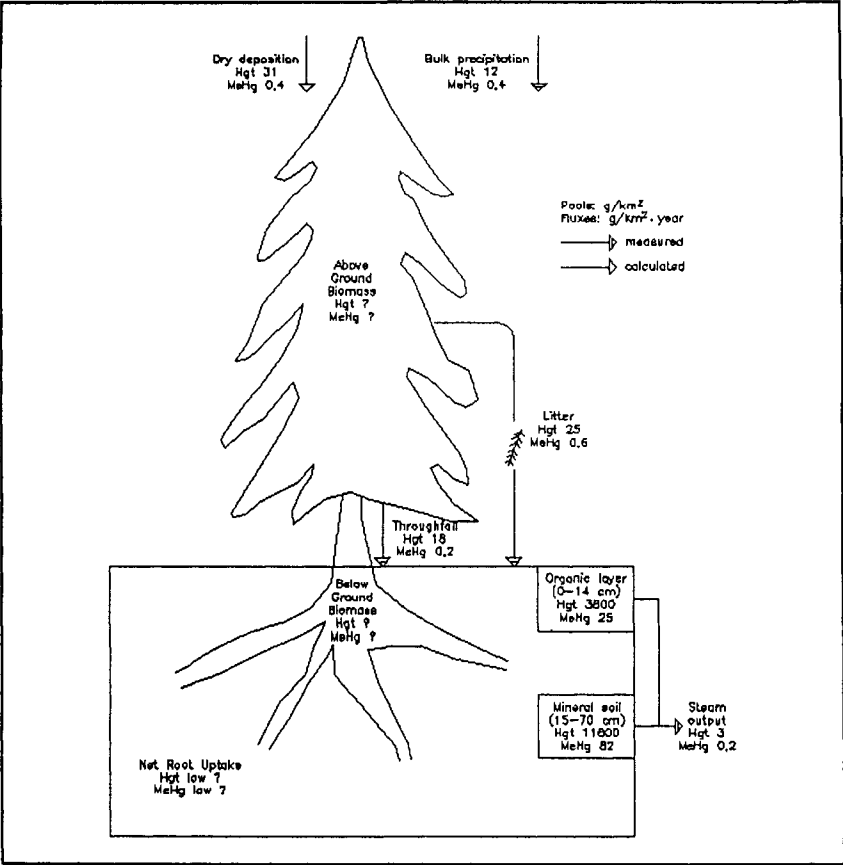


Figure 1b: New perception of the mercury cycle (adapted from [10-13])

In other words, mercury speciation analysis should be done to assess the direct influence of the individual compounds on the environment, to predict the transport behaviour in the various compartments, and to develop an approach for the decontamination of polluted areas.

During the last three years, most of the experience on the determination of methyl-mercury has been gathered in connection with the development of new derivatization methods. Furthermore, valuable experience has been obtained from a recent certification exercise carried out by the EC Community Bureau of Reference (BCR, now Measurements and Testing Programme). This chapter will focus on these two issues, starting with the outcome of the BCR certification exercise which is discussed in paragraph 13.1.

13.1 Means of validation

Interlaboratory studies and the use of certified reference materials (CRMs) are both valuable tools to validate an analytical method. During recent years intercomparisons of mercury and/or methyl-mercury in various matrices have been carried out by the International Council for the Exploration of the Sea [14], the International Atomic Energy Agency [15] and the BCR [16]. Spare material from these inter-comparisons can and has been used by several laboratories for method validation [17-19]. However, these materials are usually not as well as characterized as a certified reference material and, as only statistical criteria are used to establish the reference values, the confidence interval of the mean is relatively high (greater than 10 %). Fortunately, reference materials with certified content of methyl-mercury have been produced by the National Research Council of Canada, *i.e.* TORT-1 (lobster hepatopancreas), DOLT-1 (dogfish liver) and DORM-1 (dogfish muscle); the certified contents of methyl-mercury in these materials (expressed as mass fractions of mercury) are $0.128 \mu\text{g.g}^{-1}$, $0.080 \mu\text{g.g}^{-1}$ and $0.731 \mu\text{g.g}^{-1}$, respectively. Recently, the BCR has also certified two tuna fish samples for their contents of total and methyl-mercury with certified values (expressed as mass fractions of Hg and methyl-Hg) of $2.85 \mu\text{g.g}^{-1}$ total Hg and $3.04 \mu\text{g.g}^{-1}$ methyl-mercury (CRM 463), and $5.24 \mu\text{g.g}^{-1}$ total Hg and $5.50 \mu\text{g.g}^{-1}$ methyl-mercury (CRM 464)[16]. The two BCR tuna fish samples are the result of a 5-year project which was organized in parallel with the successful work of the BCR-group on CBs [20].

The BCR project was carried out by a number of European laboratories, most of them experienced in the determination of methyl-mercury. The work consisted of a series of inter-comparisons the aim of which was to improve the quality of the analyses performed by the laboratories in a collaborative, mutual learning process. During these exercises, the laboratories gradually improved their performance until a satisfactory accuracy and precision could be obtained. The project was concluded by the certification of the above mentioned materials (CRMs 463 and 464). The stepwise procedure used by the BCR may also be recommended for individual laboratories in the validation process of their method.

The samples analyzed in the inter-comparisons reflected the systematics of the project, *i.e.* to assess the different step of the analytical chain, starting with the final detection over separation, clean-up and terminating with the extraction.

The type of sample used in the inter-comparisons is shown in Table 1. All samples were subjected to homogeneity and stability tests. After each inter-comparison, the results were thoroughly discussed amongst the participants to identify reasons for possible inaccuracy.

Table 1: Type of samples used in the intercomparisons within the BCR project on the improvement of the determination of methyl-mercury in fish [16]
* a tuna fish sample and a mussel sample were also analyzed by some laboratories in the third intercomparison

1st intercomparison	2nd intercomparison	3rd intercomparison*
a mixture of methyl-mercury and inorganic mercury in water	a raw toluene extract of a flounder	a raw toluene extract of a cod
methyl-mercury in toluene	a methyl-mercury spiked raw extract of a flounder	a method and reagent blank solution
methyl-mercury, ethyl-mercury and phenyl-mercury in toluene	a cleaned extract of a flounder	a calibrant solution
	a new mixture of methyl-mercury and inorganic mercury in water	a methyl-mercury spiked extract of a cod

The general results of the inter-comparisons as well as the homogeneity and stability testing of the materials is described in details elsewhere [21]. During this project, considerable experience was gained and this will be discussed in the following sections.

13.1.1 Extraction

The extraction method for methyl-mercury, as developed by Gage [22] and modified by Westö [23], is still widely used. The extraction is based on the addition of acid (hydrochloric, hydrobromic or hydroiodic) to a homogenized sample, the extraction of the methyl-mercury halide into an organic solvent (benzene or toluene), purification by stripping with a thiol compound (cysteine or thiosulphate) and re-extraction into benzene. The extraction method developed for packed column gas chromatography is still warranted by the use of rather unspecific detection methods like electron capture employed by several laboratories. A problem with the extraction method is the formation of often persistent emulsions which can be avoided by the use cysteine impregnated paper instead of a cysteine solution [24]. The modified Westö extraction method has also been used in combination with capillary gas chromatography [18,25], high performance liquid chromatography [26], radiochemical neutron activation analysis [27] and cold vapour atomic absorption spectrometry [28,29]. With more specific separation and/or detectors the extraction method can be simplified. For methods where the analyte is detected in an aqueous solution the re-extraction step can of course be omitted, e.g.

in a method where methyl-mercury is determined by enzymatic conversion to methane [30], or in the purge and trap gas chromatography Fourier transform infrared spectroscopy [31]. The purification process with cysteine or thiosylphate can also be substituted by using an anion exchange column (Dowex 1 x 8, Cl-form) [32].

Totally different extraction methods can be found in many new methods using derivatization and hyphenated methods. Bulska *et al.* [33] used leaching into hydrochloric acid, neutralization with sodium hydroxide, addition of borate buffer followed by sodium diethyldithiocarbamate and toluene in their butylation method. KOH/methanol dissolution of the sample was also carried out prior to sodium tetraethylborate derivatization method followed by cryogenic GC AAS [19,34].

Irrespective of the extraction method used, the extraction recoveries should be verified. This can be done by spiking a sample of similar composition to the sample analyzed with a known content of the methyl-mercury. The spiked sample is left to equilibrate and the methyl-mercury content is determined. The main drawback is that the spike is not always bound in the same way as the naturally occurring methyl-mercury. It is recommended to let the spike equilibrate at least overnight.

The verification of extraction recovery is especially crucial in speciation analysis. In the case of methyl-mercury, variation even within sample type can be substantial [35] and may be due to differences in the samples which would normally not be noticed in *e.g.* total mercury determinations. Consequently, one should be careful when trying to apply one well-proven extraction method to other sample types. Indeed, Stoeppler [32] noticed higher results when applying an ion-exchange method developed for biological samples to rain water and soil samples. It should be noted that this bias was discovered by comparison between two methods in the same laboratory. Therefore, in speciation analysis it is important to have access to at least two methods either in-house or in collaboration with another laboratory.

13.1.2 Separation

In traditional packed volume analysis it is necessary to condition the column by, *e.g.* repeated injection of mercuric chloride [36,37] or initial treatment of the column with LiCl. Still, peak tailing and adsorption of variable amounts of the organo-mercury compounds tend to give results with a rather high standard deviation. Although capillary columns are a better alternative, their use is still not very widespread. The use of capillary columns has been reported in the literature [17,18,38-40]; nevertheless, it has often proved difficult to adopt the methods described probably due to interactions between the compound and metal surfaces at high temperatures, insufficient deactivation of the glass/fused silica columns, or the choice of the stationary phase. Kato *et al.* [17], in their method (capillary column gas chromatography with inductively coupled plasma atomic emission spectrometric determination) have found that peak intensity falls and peak broadening increases directly as a function of column length and carrier gas velocity. Therefore, they used a short (3m x 0.35 mm i.d. chemically bonded fused silica) column and 7.5 ml.min⁻¹ flow rate; they also found leading and tailing of the peak when the column temperature was above 160 °C. Bulska *et al.* [33] observed substantial interactions of organomercury chlorides with both polar and non-polar, bonded-phase, fused-silica columns; they therefore proposed to form non-polar dialkyl derivatives with superior chromatography characteristics, *i.e.* by butylation with a Grignard reagent.

Petersen and Drabaek [25] have examined the use of a commercially available CP-SIL 8 CB capillary column and found that the best results were obtained using on-column injection and high film thickness.

Due to the many problems with column interaction in gas chromatography, several laboratories have been using high-performance liquid chromatography (HPLC) as an alternative. Although not employed in the BCR project, HPLC offers several advantages compared to gas chromatography: The separation of the compounds is performed at ambient temperature, thus decomposition reactions are unlikely, and the sample preparation is usually simpler. The UV detection of methyl-mercury is, however problematic but can be accomplished, *e.g.* by derivatization with organic complexing agents such as 2-mercapto-benzothiazole [41] or 2-mercaptoethanol [26,42]. HPLC seems to offer greater difficulties to a laboratory not specialized in this kind of analysis than GC. The reason for taking it up is often caused by the wish to look at other organic mercury compounds than methyl-mercury, *e.g.* the less volatile or non-volatile species like mersalylic acid or the aromatic organomercurials, which are usually difficult to determine by GC.

13.1.3 Derivatization

Another way to overcome the problem of the poor chromatographic stability of methyl-mercury compounds is to use a derivatization technique. These procedures are quite new and have been covered extensively in section 13.2. In the BCR exercise, successful results were obtained using ethylation [34], hydride generation [31] and butylation [33]. All these methods involved hyphenated techniques and were in general quite complicated. As the derivatization reactions are not always fully understood and are not well controlled, the introduction of another analytical step in the speciation procedure should be carefully considered. Consequently, the derivatization techniques can only be recommended for laboratories experienced in both these procedures and in hyphenated methods, and the laboratories should, in addition, have access to at least one other well proven method for methyl-mercury detection.

13.1.4 Detection

At the start of the BCR project, many participants used packed column GC with electron capture detection. Some participants were experienced with capillary GC and only few laboratories determined methyl-mercury routinely. Even fewer participants mastered alternative methods with element specific detection. Most likely due to the possibilities for, *e.g.* column interactions and the difficulties in obtaining well conditioned GC columns the spread in the first intercomparison results were unsatisfactory and the lack of good alternative methods made it difficult to discover the reasons for the inaccuracy. Later, hyphenated methods with element specific detectors were introduced in the interlaboratory study and the accuracy and precision gradually improved [21]. Not surprisingly, it was shown that GC methods with unspecific detection like electron capture detectors were accurate when used by experienced laboratories, while GC methods in combination with other procedures than the Westöo extraction method proved more difficult to master by new participants in the exercise.

The hyphenated methods successfully employed were cryogenic GC-AAS [34], cryogenic GC-FTIR [31], GC-MIP [33], GC-AAS [43]. These techniques which all used derivatization, are described in more details in section 13.2. Head space GC-MIP [44] and flow injection CVAFS after separation/enrichment on a microcolumn of suphydryl cotton [45] were also used in the interlaboratory study.

ICP-AES and ICP-MS can be used on-line after HPLC [46-48] but the instrumentation is usually too expensive to be used alone for organic mercury determinations. The use of capillary GC-ICP-AES has also been reported [17].

13.1.5 Primary calibrants

General aspects of calibration should also be applied to speciation analysis. Primary calibrants should be used and calibrant solutions should be verified either by using two independent solutions or by verifying the new calibrant solution with the previous one.

In speciation analysis, the matrix effects can be even greater than for total determinations and variations can be encountered within the same sample types as discussed above. For this reason, it is recommended not only to validate the methods for each type of matrix and for the extraction agent applied, but also to perform standard addition on every sample. It should be stressed, however, that standard addition requires an evaluation of the linear range of the detector response.

Ethyl-mercury has been used as an internal standard in GC methods [18,29]. Petersen and Drabaek [25], however, showed that the use of ethyl-mercury did not always improve the precision in GC methods due to possible interactions with the column.

13.2 Critical review of derivatization methods

Analytical speciation implies that more assumptions are made in comparison to determination of total element contents, which means that the verification of the accuracy is much more complicated. Methods usually used for the determination of methyl-mercury consist of two main parts, isolation and detection; in addition, a varying number of clean-up steps have been also applied as described in section 13.1.

Several new methods combine the isolation step as a sample pre-treatment with a derivatization step. Generally, additional steps in an analytical procedure increase the possibility for matrix effects of which many may be left undetected in the isolation/derivatization steps. Sample pre-treatment may induce errors such as incomplete extraction, species transformation or losses. Additional errors which may occur at the derivatization step are destruction of the derivatizing agent, incomplete transformation of the species of concern and decomposition of the often very reactive derivate. The type of isolation applied, *i.e.* the combination of pre-treatment and derivatization steps, is often critical to achieve an efficient and accurate derivatization of methyl-mercury in the matrix of concern. It should, therefore, be stressed that the verification of the isolation procedure should always be performed with great care. Cross-laboratory verification of various isolation/derivatization procedures, as well as use of the standard addition technique, are essential tools to verify the accuracy of the determination, especially if a derivatization step is part of the analytical procedure. Consequently, the use of derivatization techniques can only be recommended to laboratories experienced in methyl-mercury determination and having at least one alternative method of proven

performance for the methyl-mercury detection.

Nowadays, the most common derivatization technique applied for the detection of methyl-mercury in environmental samples is based on ethylation (generation of volatile methyl-ethyl-mercury species). Based on the use of sodium tetraethylborate $[\text{NaB}(\text{C}_2\text{H}_5)_4]$ as a suitable reagent for the ethylation of methyl-mercury [49], Bloom [50] developed a method for the determination of methyl-mercury in fish. This method has been adapted and used by several research groups and has also been critically evaluated for other types of matrices, e.g. sediments, soils and natural waters [19,34,51-53]. However, the use of other derivatization reagents has also been successfully tested, e.g. using NaBH_4 or $\text{LiB}(\text{C}_2\text{H}_5)_3\text{H}$ [43], or butylation with a Grignard reagent [33]. This section will mainly discuss on the use of ethylation as derivatization procedure.

Ethylation of methyl-mercury in environmental samples usually requires an initial sample pre-treatment step in order to separate methyl-mercury from the matrix and to avoid serious interferences in the ethylation process. It is obvious that different matrices will cause more or less severe interferences. For example, water rich in organic matter will generate high levels of interferences if ethylation is applied directly; organic ligands, especially with a sulphur donor atom, may also strongly interfere in the ethylation process [34]. The sulphide ion is probably the most serious interferent, independently from the type of matrix (e.g. sulphidic sediments or anoxic waters) [50-52]. Lee *et al.* [34] also reported a lower ethylation efficiency for the analysis of water containing strongly complexed methyl-mercury species. This reduced efficiency related to the presence of humic acids has been demonstrated in a method inter-comparison [53].

Recently, pre-treatment procedures have been further developed to minimize matrix interferences on the ethylation of methyl-mercury. Alkaline digestion of fish and sediment samples with a methanolic potassium hydroxide solution has been reported to be a suitable pre-treatment technique prior to ethylation for methyl-mercury determination [19,50]. As discussed above, the method has, however, serious drawbacks when applied to sulphidic sediments. In addition, an excess of inorganic mercury in comparison to methyl-mercury, along with an ethylation reagent of poor quality, may yield to an accidental formation of methyl-mercury from the inorganic mercury substrate [51].

As an alternative to the alkaline digestion method, a procedure based on distillation of methyl-mercury from biological and other environmental samples has been reported [54]. The main advantage of this procedure was that matrix effects from sediments on the ethylation efficiency were avoided [51].

The distillation pre-treatment of the sample is also applicable to the analysis of various types of waters. For the determination of methyl-mercury at the sub-picogram per litre level, Bloom [50] developed a technique based on extraction with methylene chloride followed by back-extraction into water via a solvent evaporation step. However, besides the formation of emulsion during the extraction step of rich-in-humic acid water [34], low recoveries and less reproducible results have been reported for anoxic/sulphidic waters as well as for natural waters with high organic matter content [34,52,53]; this resulted from interferences during ethylation and, to some extent, to an incomplete release of methyl-mercury from strong complexes. Problems in determining methyl-mercury in water rich in humic substances have also been reported for another procedure, involving pre-concentration onto a dithiocarbamate resin-loaded micro-column followed by butylation with a Grignard reagent [55]; the explanation was not clearly established in this later

case, and problems could be related to a low column efficiency, but possible interference effects on the derivatization was also suspected. These interferences may be avoided by using the distillation technique which allows an efficient separation of methyl-mercury from complex natural water matrices to be achieved [34,52,53].

13.3 Advice on how to undertake Hg speciation analyses

In starting up a new determination procedure for methyl-mercury several aspects have to be considered by an unexperienced laboratory. First, it is always recommended that a good reliable method for total mercury determination is available within the laboratory. In the case of fish samples, the total mercury content is often, but not always, a good approximation of the methyl-mercury content. Also, it is recommended that the method for total mercury determination is as sensitive as the intended method for methyl-mercury determination. Secondly it is important to consider the type of matrices that will be introduced in the analytical procedure. Matrices like tuna fish do not pose hard demands on detection limit, unlike to planktons which generally contain low levels of methyl-mercury. It is also important to know whether the goal is to obtain a good indication of the percentage of methyl-mercury in the sample, or if the analysis is part of a pathway study. In the latter case, a discrimination between different organic mercury forms is often necessary. According to the matrix, very roughly speaking, the determination of methyl-mercury can be classified into the three groups depicted in Table 2.

Table 2: Classification of the application of methods for methyl mercury determination according to complexity.

	High content of methyl mercury	Low content of methyl mercury
Indication of percentage methyl mercury in the sample	I. Simple	II. Advanced
Pathway/metabolism studies	II. Advanced/ III. Sophisticated	III. Sophisticated

In the case of a simple application (I), it is recommended to start with total organic mercury determination using an extraction method and, e.g. AAS or GC-ECD. For the advanced application (II), the total organic mercury determination can still be applied, but it is recommended to have a more specific determination of methyl-mercury, e.g. by using capillary column GC-ECD. In pathway/metabolism studies, however, the need for the combination of low detection limit with specificity and determination of other organic mercury forms means that more sophisticated methods (III) have to be applied, e.g. hyphenated methods and derivatization techniques.

In all cases it is recommended, and in the case of the advanced and sophisticated applications it is indispensable to compare the method applied with an independent method either within the laboratory or at another laboratory.

Available CRM's should always be used. In the case of speciation analysis it is important to remember, however, that different matrix effects can be encountered even between the sample types of similar category (*e.g.* fish sample).

13.4 Conclusions

Speciation of mercury is considered necessary to understand various pathways of mercury in the environment, to make correct assessments of toxicity of specific mercury compounds, and to be able to develop relevant strategies for decontamination of polluted areas.

When starting up a determination procedure for methyl-mercury in biological samples a general stepwise approach is recommended, *i.e.* to assess the different steps in the analytical chain, starting with the endpoint of the procedure, the final detection, moving backwards over separation, cleanup and finally the extraction.

From the experience gained in certification exercises it has been concluded that:

- whatever the extraction method used the extraction recoveries should always be verified, *e.g.* by standard addition using prolonged equilibration times. The verification is especially crucial in speciation analysis. At least for methyl-mercury, variation within sample type can be substantial, perhaps due to differences in the samples normally not noticed, *e.g.* in the total mercury determination procedure;
- GC methods can pose many problems due to interactions with the column. It does, however, provide a good starting point for methyl-mercury determination in *e.g.* fish samples, but high accuracy can normally be achieved only by experienced laboratories;
- due to the many problems with column interaction in GC, HPLC can be applied as an alternative. However, experience in HPLC techniques is required;
- an alternative to overcome the problem of the poor chromatographic stability of the methyl-mercury compounds, is to use a derivatization technique; it should be recalled, however, that derivatization is prone to possible interferences and should be carefully applied;
- in speciation analysis, influence from the matrix can be even greater than for normal total level analysis. As mentioned earlier, variations can be encountered even within the same sample types. For this reason, it is recommended not only to validate the methods for each type of matrix and for the extraction agent applied, but also to perform standard addition on every sample.

When starting a technique for the determination of methyl-mercury determination in a laboratory, considerations should be given to (i) the sample type (level of methyl-mercury in the sample and percentage of mercury as methyl-mercury), and (ii) whether the plan is to perform pathway studies or if the objective is to find out the amount of methyl-mercury in the sample. It is important to be able to determine the total mercury content with a reliable technique available in the laboratory. A good way to approach mercury speciation is to start with e.g. a simple GC method, to optimize it and use capillary columns to get more specific determinations and better detection limits (alternatively HPLC methods can be used). For more advanced/sophisticated applications like pathway studies, hyphenated techniques and derivatization methods should be considered. It is always necessary to verify the performance of the method newly installed in the laboratory by comparing it with another method and to use available CRMs.

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14.

Speciation analysis of organolead compounds. Status and future prospects

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The pollution of the environment by lead is ubiquitous and has a global character. The major source of this element in the environment is the combustion of leaded gasoline. Despite severe restrictions in force in many countries, the use of tetraalkyllead as an antiknock agent remains the largest industrial application of organolead compounds and represents about 5-7 % of the total lead consumption, which is estimated at 3-3.5 M tons [1,2]. Other applications of organolead are less important and include the use of tetraethyllead (Et₄Pb) in the manufacture of ethylmercury while trialkyllead compounds have been used as wood preservatives, antifouling agents in marine paints, and components of pesticides [1]. Evidence for the environmental formation of alkyllead from inorganic lead in nature is mainly circumstantial. The amount of organolead produced by biomethylation, if any, is insignificant when compared to the anthropogenic emission [3,4].

The harmful effects of organolead compounds are considered to be much larger than those of inorganic lead [4-6]. The toxicity of alkyllead species diminishes in the sequence $R_4Pb \rightarrow R_3Pb^+ \rightarrow R_2Pb^{2+} \rightarrow Pb^{2+}$ (where R is a methyl- or ethyl- group) but the ionic forms are more persistent in the environment. In algae and higher plants alkyllead compounds were found to be responsible for the inhibition of growth, disturbances of mitosis and ultrastructural alterations. The organolead contamination of the aquatic environment is known to affect fish. About 150 fatal cases of human intoxication with Et₄Pb have been reported in the literature. They were related to accidental exposures but long term environmental exposure to low levels of organolead has been associated with a wide range of metabolic disorders and neurophysical deficits especially to children.

Starting in the seventies, the growing concern about the contribution of organolead to the lead burden of the biosphere has stimulated the development of analytical methodologies capable of discriminating between the inorganic lead - Pb(II) and traces (0.1-1 % of the total lead) of organolead - Pb(IV), and further among the different organolead species. The currently accepted approaches are based almost exclusively on the use of hyphenated techniques as discussed in a few monographs [7-9].

These techniques are based on a combination of a separation technique such as gas chromatography (GC) or high performance liquid chromatography (HPLC) with an element sensitive and selective detection technique, usually atomic absorption or emission spectrometry. The use of non-specific detectors is restricted owing to the interferences of many hydrocarbons occurring at high concentrations in real samples. The only noteworthy non-spectrometric technique proposed, differential pulse anodic stripping voltammetry [10,11], shows limited application especially when applied to the analysis of real samples.

In the past 5 years, speciation analysis of organolead has been the subject of a few comprehensive review papers [4,12,13]. The very recent advances in instrumentation have rendered the routine detection of lead possible at the sub-pg level. However, severe limitations still remain on the level of sample preparation. Most of the existing procedures are very cumbersome, requiring a large amount of sample and tedious separation-preconcentration steps. Moreover, controversies still exist about the recovery of analytes and the efficiency of their derivatization which depends on the matrix type involved. Ultratrace analysis for organolead in the remote environment is likely to face the blank value problems common in trace analysis for total lead. Effective studies of pathways and natural sources of organolead require more straightforward analytical methods that are capable of the reliable determination of organolead species at concentration levels down to sub-ng.l⁻¹ in waters and sub-ng.g⁻¹ in biological materials or sediments. Because of a large variety of sample types, custom-tailored methods need to be designed.

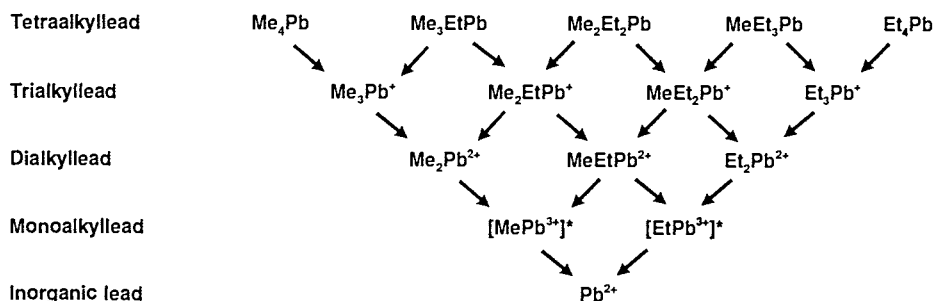
This chapter aims to evaluate the state-of-the-art of methods available for the speciation analysis of organolead in different samples. Advantages and limitations of the analytical procedures applied are critically discussed with respect to sample preparation, chromatographic separation and detector operating conditions as well as the chromatograph-spectrometer interface design. Very recent developments in speciation analysis for organolead by capillary gas chromatography, atomic emission and mass spectrometry are highlighted. Particular attention is given to the factors affecting accuracy of analyses and to the validation of the results.

14.1 Sources and concentrations of interest of organolead compounds

14.1.1 Atmosphere

Atmospheric pathways play a predominant role in the biogeochemical cycling of organic lead [4,14,15]. The presence of organolead in air is mainly due to the release of unburnt tetraalkyllead from motor vehicles through exhaust or by evaporation from the fuel tank and carburettor. Other sources are associated with the manufacture, transport and handling of leaded gasoline at filling stations resulting in direct evaporation, accidental spillages or displaced fuel tank vapours. Organolead compounds added to the gasoline are mainly tetraethyllead and tetramethyllead but some mixed methylethyllead compounds may also be involved. During combustion they undergo a variety of degradation processes *via* ionic organolead species to inorganic lead. Total alkyllead represents about 4-13 % of the lead emission from cold, choked engines which fraction drops to 0.3-3 % for hot, moving cars [16]. The emission from two-stroke engines is more significant. Di- and trialkyllead compounds may be present in high amounts in the exhaust gas.

The degradation processes continue in the atmosphere *via* photolysis or homogeneous gas-phase reaction with hydroxyl radical (OH), ozone (O₃), triplet atomic oxygen O(3p), molecular oxygen (O₂), NO₂ and SO₂. Heterogeneous reactions on the surface of atmospheric particles may also play a minor role [17]. A degradation scheme showing the possible species involved is illustrated in Figure 1. Me₄Pb and Et₄Pb were shown to be short-lived: $t_{1/2}$ = 0.6-2 h and 5-10 h, respectively while their direct decomposition products, Me₃Pb⁺ and Et₃Pb⁺, are a factor of 3-5 more persistent [18]. These values are laboratory estimates while under real high tropospheric conditions, especially over the Arctic, the species seem to be more persistent [19]. There is a lack of unambiguous evidence for the environmental occurrence of RPB³⁺ as this compound is apparently very unstable with a short half-life.



* very unstable, evidence for the presence only circumstantial

Figure 1: Organolead compounds used as petrol additives and products of their degradation.

14.1.2 Air gaseous phase

There are wide discrepancies among the organolead atmospheric concentrations reported. One reason for this is certainly the doubtful reliability of many analytical procedures used, especially those not involving a chromatographic separation.

The range of concentrations reported for gaseous phase organolead in the urban, semiurban and rural environment are summarized in Figure 2. The largest concentrations are measured near filling stations, parking areas and in city centres while a distinct drop of the concentration of organolead with increasing distance from urban regions is observed. No data are available yet on the concentration of organolead in air in the very remote, viz. polar atmospheres.

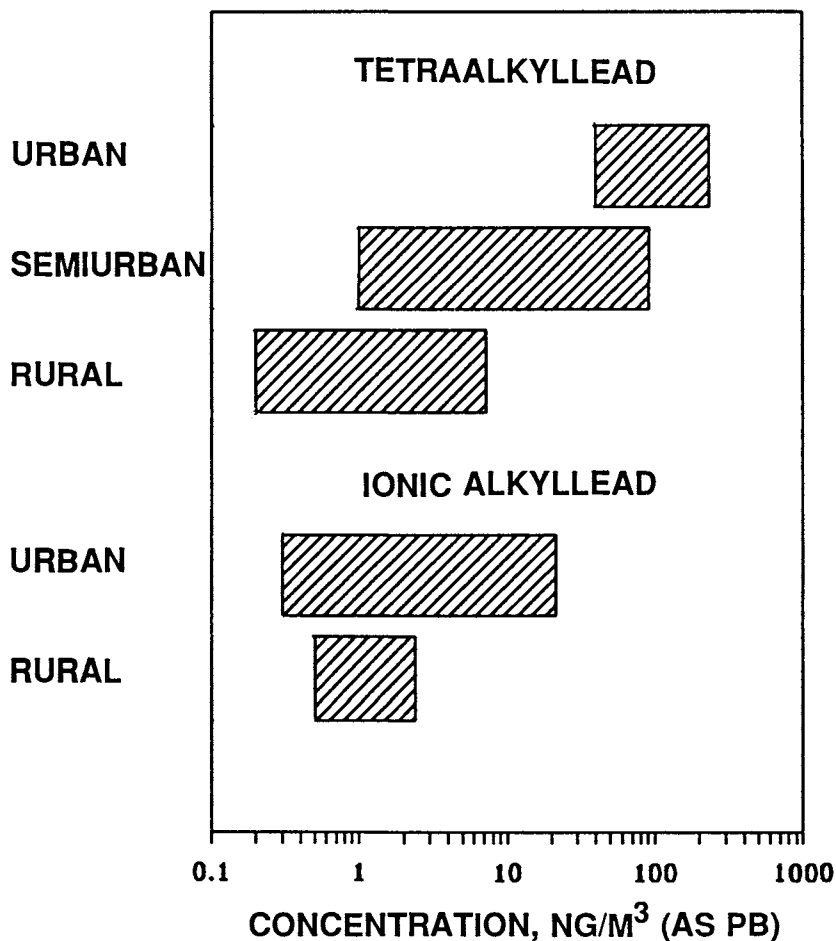


Figure 2: Range of organolead concentrations in air in: urban (A), semi-urban (B) and rural (C) environment

The typical alkyllead/lead ratio in the air gaseous phase is 1-2 % and correlates strongly with inorganic lead at the urban site, indicating an automotive source [20]. Air samples taken at rural sites show a ratio of 30-40 %, which suggests the influence of air masses in which sink processes have removed alkyllead and inorganic lead at different rates [17,20,21]. In addition, a natural environmental alkylation process as a source of atmospheric vapour-phase alkyllead was suggested as being responsible for a large number of elevated ratios in the air that had passed over the open sea or estuarine and coastal areas [17,22]. The hypothesis of the natural origin of organolead in maritime air needs to be supported by the proof that methylleads are the species

actually occurring. A recent species specific study of organolead in Greenland snow showed that none of the oceanic air masses in mid-summer, at the apogee of ocean bioproductivity, gave rise to a snow deposit with a detectable concentration of methyllead. On the contrary, methyllead species were abundant in winter and spring snow on having arrived *via* long range advection from Europe [19].

The occurrence of organolead in air depends on the source, the residence time of the air masses and the intensity of transformation processes. Me_4Pb and trimethyllead are ubiquitous in Europe, where Me_4Pb is the dominant antiknock compound used, especially in winter, and account for more than 50 % of the total organolead. In the US and Canada where Et_4Pb prevails, larger proportions of this compound occur although its concentration is lower than the level expected on the basis of the antiknock additive composition due to its lower stability and volatility [23].

14.1.3 Air aerosol

Relatively little is known on the presence of organolead in atmospheric aerosols [20,24-26]. Alkyllead compounds are generally found in aerosol samples at pg m^{-3} levels, some 2-3 orders of magnitude below the concentration of gaseous alkyllead compounds. Ionic compounds (especially dialkylleads) are the dominant species. The fraction of the aerosol alkyllead may increase in the remote environment, *e.g.* Arctic Haze when the breakdown of tetraalkyllead (TAL) is complete, the aerosol concentration is large and the breakdown products show higher affinity to the aerosol than to the vapour phase.

14.1.4 Atmospheric deposits

Alkyllead species may be removed from the atmosphere by dry and wet (rainout/washout) deposition processes. The concentrations of alkyllead compounds measured by species selective techniques in rainwater and snow collected at several European sites are summarized in Figure 3. Organolead compounds in rainwater are generally present at concentrations less than 1 % of the dissolved ($<0.45 \mu\text{m}$) inorganic lead.

Tetraalkyllead compounds are not found in atmospheric deposits due to their rapid degradation to ionic alkyllead in the upper strata of the troposphere. Ionic tri- and dialkyllead species are ubiquitously found in varying proportion, depending on the sampling site and date. The presence of monoalkyllead compounds reported in some cases is very likely due to artefacts of the analytical procedures used. The photochemical degradation of organolead is reflected by seasonal variations in $\text{Pb}_{\text{org}}/\text{Pb}_{\text{tot}}$ and $\text{PbEt}_3^+/\text{PbEt}_2^{2+}$ ratios with maxima in summer and minima in winter [19,27]. The differences in proportions of organolead to total lead in urban and rural atmospheric deposits are not so pronounced as in the air, probably due to different levelling processes [28]. A recent study of organolead in fresh Greenland snow showed, however, a distinct enrichment with a fraction of organolead reaching 2 % of the total lead present [19]. The analysis for organolead in the polar environment is a challenge for the analyst due to very low concentrations involved ($10\text{-}100 \text{ fg.g}^{-1}$), small amount of sample available ($< 50\text{-}100 \text{ g}$) and the unique significance of the data to study the global biogeochemical cycle of organic lead, long-range transport of petrol-related emissions and, by reading glacial records, the effect of leaded gasoline on the global tropospheric pollution during the past century [29].

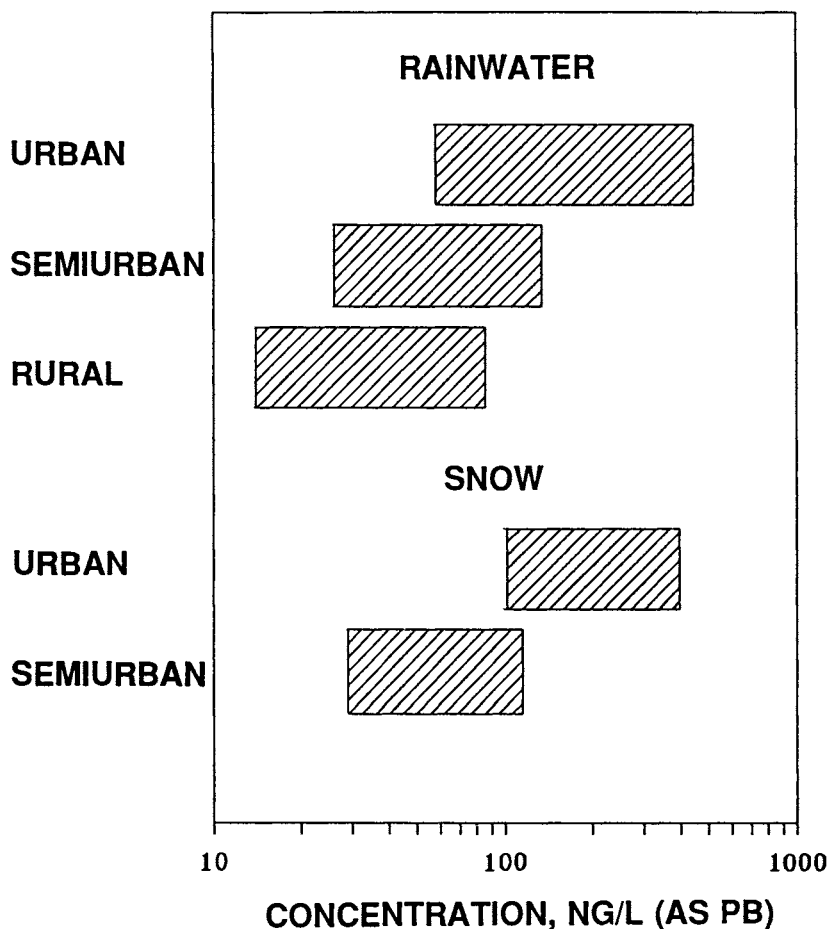


Figure 3: Ranges of organolead concentrations in rainwater in: urban (A), semi-urban (B) and rural (C) environments

14.1.5 Hydrosphere

Organolead compounds can contaminate surface waters *via* atmospheric precipitation [4,14,27], road and river runoffs [4,14,30] as a consequence of industrial and municipal discharges [4,14,31] or leaching from commercial and industrial wastes. Some accidents (*e.g.* the Cavtat one) were responsible for local pollution of aquatic reservoirs by organolead [32].

Tetraalkylleads have not been reported in surface and ground waters. Due to the non-polar nature, relatively high vapour pressure, lipophilic character and high density, R_4Pb compounds are expected to volatilize from water to the atmosphere,

partition into the lipid phase (e.g. in fish tissues), or to adsorb on sediments. In addition, tetraalkylleads entering water reservoirs are rapidly degraded abiotically and *via* metabolic processes [4,14] to result sequentially in R_3Pb^+ , R_2Pb^{2+} , RPb^{3+} and Pb^{2+} . The reverse process, an environmental methylation, remains controversial [3,5,17].

Tri- and dialkyllead compounds have been measured at $\mu g.l^{-1}$ levels in samples collected in the vicinity of a tetraalkyllead manufacturing plant [31] and at sub- $\mu g.l^{-1}$ levels in road drainage and surface waters [30]. In river water normal levels in the absence of a proximate pollution source appear to be at low $ng.l^{-1}$ [33] or sub- $ng.l^{-1}$ [34] levels. In seawater, lake water and ground water organoleads have usually been reported at concentrations below the detection limits ($0.1 - 0.3 ng.l^{-1}$) [14].

14.1.6 Sediments, soil and dust

In aquatic systems, sediment is the ultimate storage sink for organolead compounds. Alkyllead compounds are bound to sediment either physically (by adsorption) or chemically (by complexation) and can become available to biota through leaching and/or ion-exchange. Sediment was considered to be a suitable medium for biomethylation of $Pb(IV)$ [17,21]; such a phenomenon is, however, not likely to be occurring for $Pb(II)$.

Hitherto, the determination of organolead in sediments has been restricted to few "illustrative" samples and systematic environmental studies have not yet been undertaken. Considerable concentrations of organolead compounds were found in sediments sampled close to a manufacturing plant and in samples collected near the wreckage of the M/S Cavtat. Lake and river sediments normally show sub- $ng.g^{-1}$ amounts of alkyllead [14,34]. Contents of $0.04-0.07 ng.g^{-1}$ were found in some North Sea sediments [34]. Content at a level of $10 ng.g^{-1}$ was measured in street dust [4,14]. Various aspects associated with alkylleads in soils were recently reviewed [5].

14.1.7 Biological materials

Organolead compounds have been determined in grass and tree leaves [35], wine grapes [36], plant sap [37] and some vegetables [38].

Studies on invertebrate fishes have been carried out to investigate the presence of organolead [39]. A typical concentration of organolead compounds in fish in unpolluted areas is estimated to be in the low $ng.g^{-1}$ range [40,41]. The results reported for fish caught in the proximity of pollution sources reach values 3-4 orders of magnitude higher, indicating the accumulation of organolead in fish [42], especially in gills, intestines and liver.

Me_3Pb^+ is more abundant in mussels than Et_3Pb^+ . The highest contents of Me_3Pb^+ were found in the muscle, gills and visceral tissue. The distribution pattern of Et_3Pb^+ is slightly different with the highest level in the muscle, visceral mass and mantle [42].

In birds living in areas far away from any emission source trimethyllead was found to be an ubiquitous species while mixed methylethyllead species were also abundant [43].

In mammals, inhalation or adsorption of R_4Pb compounds results in the formation of trialkyllead in tissues and body fluids. With the exception of methylleads found in the blood of petrol tank cleaners [44], alkylleads are usually below the detection limit in blood samples [45-47]. The levels in human urine were also found to be below the detection limits [47,48].

14.1.8 Miscellaneous

Organolead compounds were found in tap water at levels of 0.2-5 ng.l⁻¹ [49-51]. A recent report revealed relatively high concentrations (up to 0.1 µg.l⁻¹) of organolead in wine [37]. Me₃Pb⁺ was the dominating species, indicating a possibility of biomethylation during the production process. Ethylated species were identified only in wines manufactured in industrialized zones.

14.2 Sample handling prior to measurement

All the techniques proposed for the determination of organolead require a more or less complex sample preparation step. This is due, on the one hand, to extremely low concentrations of these pollutants in the biosphere, which are not matched by the detection characteristics even of the state-of-the-art instrumentation, and which thus make an enrichment step mandatory. On the other hand, the complex nature of some types of samples, such as biological materials or sediments, renders the separation of the analytes from the sample matrix necessary before a sample may be introduced on a chromatographic column. An additional step involving the conversion of the analyte to a form amenable to the analytical technique applied (derivatization) is often required. Different approaches are needed depending on the character of the analyte (non-polar or ionic compounds) as well as the nature of the sample: gas, aerosol, water, sediment or biological material. A general layout of the sample preparation sequence is shown in Figure 4.

14.2.1 Sampling and Storage

Many errors may arise at an early stage of the analytical procedure (sample collection and storage) but little is known on the effect of particular factors on speciation analysis of organolead. Precautions to avoid contamination from automotive sources has to be taken during sampling, especially in the remote environment. Generally, sampling and storage protocols recommended for the analysis of the particular type of the sample for trace metals or volatile organic contaminants should also apply for organolead. Some specific requirements are discussed below.

14.2.1.1 Air

Early methods of sampling were usually based on chemisorption using scrubber solutions such as, e.g. iodine monochloride (ICl) in dilute hydrochloric acid, iodine (I₂) in a KI solution or solid reagents (I₂). During scrubbing tetraalkyllead species were quantitatively converted into alkyllead salts which were further determined using, e.g. the dithizone method. These techniques were not selective with respect to organolead and the subsequent determination was often subject to interference by inorganic lead resulting in doubtful accuracy of the analysis.

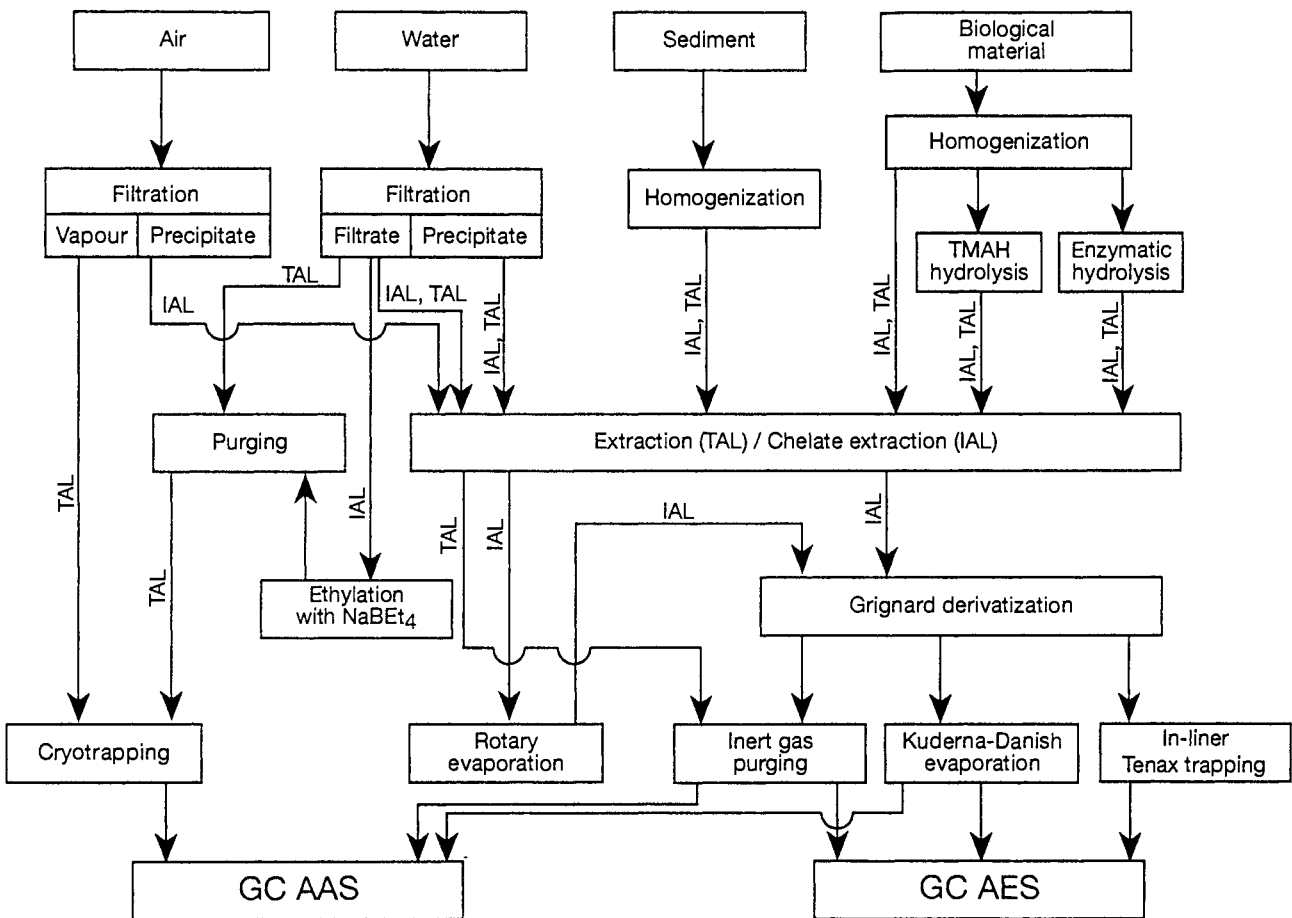


Figure 4: Layout of the sample preparation scheme for speciation analysis of organolead

In modern analytical methods sampling of air is usually integrated with an enrichment step. Organolead species are determined in the gas phase and in the particulate fraction. For the separation between organic and inorganic lead in air, filtration is usually the method of choice. Due to relatively poor sensitivity of the available detectors large volumes of air (100-1000 m³) have to be sampled which are pumped through a 0.45 µm filter. The particulate material, assumed to contain virtually all the inorganic lead and most of the ionic organolead, remains on the filter while the organic lead vapours pass through the filter for the enrichment. The techniques used include cryocondensation [15,18,24] and sorption, usually on porous polymers [23,52-54].

The universal character of cryotrapping for the concentration of atmospheric trace contaminants has the side effect of risk of clogging of the trap by water at the low temperatures used. Chemical drying agents tend to remove part of the organolead from the air stream. A cryogenic pretrap (e.g. an empty tube kept at -15 °C [55] or a washing flask kept at -78 °C [56]) may be a remedy but the loss of ionic trialkyllead and tetraethyllead is a real hazard [57]. An important problem may constitute reactive photochemical oxidants, e.g. ozone or peroxyacetyl nitrate, which are collected together with R₄Pb at low temperatures and may cause decomposition of R₄Pb. The risk of breakdown of tetraalkyllead species during sampling can be avoided by the removal of the oxidants from the air stream prior to collection. A filter (a Teflon tube packed with FeSO₄) attached to the upstream end of this to remove atmospheric aerosol was proposed. Such a filter seems to be a valid approach for TAL but trialkylleads are partly removed [58]. Despite recent advances in the design of a cryotrap, practical difficulties are associated with the use of this concentration technique for sampling in remote areas which hamper its use in semiautomated sampling. Solid adsorbents are an alternative of a continuously growing interest. XAD [23], Porapak® [52] and Amberlite® [23] resins and Tenax® [53,54] have been studied. A severe degradation of TAL on the absorption medium in particular was observed [52]. Once in the trap organolead compounds are easily transferred into solution: R₄Pb into hexane and R₃Pb⁺ and R₂Pb²⁺ into water [24]. When ionic alkylleads are not of interest the cryotrapped or sorbed TALs can be transferred to the measurement system by thermal desorption.

Much work still needs to be done to assure the reliability of sampling gaseous atmospheric organolead. A valid collection method must consider the presence of ionic organolead in the gas phase. Decomposition during sampling may be compensated for by the use of an internal standard of deuterated Me₄Pb and Et₄Pb [52]. Difficulties with the proper addition of this standard to the sample prior to trapping were observed. In addition the deuterated standard can only be useful for MS and probably atomic emission analysis.

14.2.1.2 Water samples

The initial cleaning of the containers is very important to avoid contamination especially if the same items are used for samples with different analyte concentrations. A very efficient way of cleaning consists in a simple soaking of the glassware in hot concentrated HNO₃. The sampling protocols extensively described in the literature for ultratrace analysis can usually be applied. An acidification of samples is recommended to prevent losses by adsorption on the container walls.

Unlike what occurs for inorganic lead this process does not seem to change the speciation of organolead. Preconditioning with the sample solution has to be avoided to prevent adsorption and accumulation of organolead compounds on the container surface [30,58]. Instead, after being emptied the container is treated with a portion of an organic solvent in order to release adsorbed organolead species which are then added to the extract.

The need for filtration prior to analysis is dependent on the level of particulate suspended matter in the sample. In unfiltered samples changes in the distribution of organolead may occur with time due to adsorption and desorption processes at the particle surface. Filtering ensures homogeneity of the analyzed sample but may lead to losses of volatile tetraalkylleads [42]. In general, it is recommended to filter samples with high particulate load such as urban deposits, river, estuarine and shallow coastal waters and eutrophic lakes; it can be avoided for sea water, tap water and atmospheric deposits from remote environments.

The preservation of analytes in their initial state prior to analysis may present difficulties. TAL compounds are likely to adsorb onto glass walls [28,59] then decomposing on the active sites [60]. The ionic organolead (IAL) species are not adsorbed to an appreciable extent [28]. Organolead compounds are known to decompose in solution in a light-induced process promoted by microorganisms, suspended solids and various impurities [28,60,61]. TAL species are the least stable and degrade completely within a few days [28]. Methyllead species are more stable than ethyllead and dialkyllead compounds are more stable than trialkyllead. Generally, no noticeable change of IAL species in water samples stored in glass bottles at 4 °C in the dark was observed for a period of up to 1 - 3 months [28,60,61]. It must be emphasized that decomposition rates depend on the origin and composition of sample and very little is known on the stability of dilute solutions (below 10 ng.l⁻¹).

14.2.1.3 *Sediments and soils*

Particulate matter suspended in natural waters can be collected by filtration using a 0.45 µm filter. For representative samples, however, very large volumes should be filtered which leads to a risk of filter clogging. Therefore, continuous-flow centrifugation is recommended.

Relatively little work has been devoted to the behaviour of organolead in suspended matter and sediments during storage. General protocols for trace metal analysis of sediments are assumed to apply for organolead. Keeping soils and sediments at room temperature in a desiccator for 1 h and sieving through a 60 mesh size screen before analysis is recommended [62]. Freezing of anoxic sediments was shown to cause very little change in the fractionation pattern for total lead analysis [63] and may be a useful storage procedure. Stability of organolead during storage is, to a large degree, unknown. Me₃PbCl was found to be rather unstable in sediment and soils from relatively pristine sites; it is degraded to Pb(II) by both chemically and microbially mediated processes. An environmentally mediated methylation is often postulated to counteract this degradation route.

14.2.1.4 *Biological materials*

Protocols recommended for the determination of ultratraces of metals in biological materials also apply to speciation analysis for organolead. However, the need for rapid analysis or freezing of the samples should be emphasized [64]; if not applied, the enzymatic activity and natural proteolysis and autolysis processes will continue after sampling and can alter the speciation. The lipophilic character of organolead compounds favours their accumulation in particular tissues of living organisms and stresses the need for a dissection of the parts of interest and homogenization prior to analysis. Sample tissue or plant material can be pulverised in liquid nitrogen to break up the matrix. Blood samples were hemolyzed by freezing (-20 °C) for at least 24 h [65]. Storage for extended periods under such conditions was found not to affect the stability of organolead initially present. Storage of samples in daylight should be avoided due to a hazard of considerable losses especially of ethyllead species [50].

14.2.2 *Extraction*

Extraction is the most widely used technique for the separation of organolead from the sample matrix. Tetraalkyllead species are quantitatively extracted from water saturated with NaCl into a 20 times smaller amount of hexane [66]. A similar preconcentration factor can be obtained by twice repeated extraction from a hydrolysed fish homogenate [67]. The characteristics of di- and trialkyllead species do not allow them to be readily extracted by any organic solvent. Me_3PbCl and Et_3PbCl may be transferred into hexane after saturation of the aqueous phase with NaCl [68] but only for Et_3PbCl is a reasonably high extraction efficiency obtained. The chelating agents assisted extraction gives far better results. Dithizone, diethyldithiocarbamate (DDTC) and tetramethylenedithiocarbamate (TMDTC) are most often used as reagents while pentane, hexane or benzene are used as extraction solvents.

Dithizone is apparently the least convenient chelating agent due to its non-quantitative extraction efficiency, low stability and the need of addition of polar solvents [43,64,70-72]. The separation of organolead dithizonates involves two consecutive extractions from a $\text{KCN-Na}_2\text{SO}_3$ buffer at pH 8.0 and pH 9.0, respectively, with 20 % dichloromethane in hexane; pH programming is needed as no single pH was found to be optimal. In addition, the dithizone extraction is not recommended to be performed prior to HPLC separation of ionic organolead species [71]. Indeed, liquid chromatography of dithizonates is characterised by large variations in the peak resolution and retention times for replicate injections due to the low stability of lead-dithizone complexes. The chromatographic characteristics of lead analytes consequently vary with concentration and possibly the nature of other co-extracted metals dithizonates. The unique advantage of the dithizone method is, however, the possibility of reextraction of ionic organolead into the aqueous (dilute HNO_3) phase. Using this procedure, the analytes can be extracted from the bulk of the organic matrix which might affect both the separation and the detection. Subsequently, a three-fold extraction at pH 8 with dithizone or with TMDTC in hexane transfers the organolead species back into the organic phase where they can be derivatized.

Extraction of the complexes of ionic organolead species with DDTC at pH 6-9 into benzene [42] or hexane [51,53,54,66] was found to be more efficient as only one extraction was necessary to obtain quantitative recovery. Pentane may also be used as the extraction solvent but the extraction has to be carried out at pH 9 [72]. Dithiocarbamates are not as sensitive to light as dithizonates which makes the handling easier and the procedure more reliable. Controversy exists about the possible interference of the coextracted inorganic lead with the subsequent derivatization and the determination [73]. The high selectivity of the hexane-TMDTC extraction system for ionic alkylleads over Pb^{2+} facilitates greatly the determination of these analytes in matrices contaminated with high levels of Pb^{2+} . Inorganic interferences with subsequent derivatization may be effectively masked with EDTA.

14.2.3 Preconcentration

The purge and trap method is widely used for the preconcentration of tetraalkyllead compounds. They are volatilized from the sample by purging with helium or argon gas and collected on GC-packing in a U-tube [40,41]. By heating the U-tube the species are desorbed from the packing into a GC column or directly into the detector system for analysis.

Extraction methods have the disadvantage of yielding a large volume of extract (usually about 5 ml). It oversizes considerably the amount which can be introduced onto the capillary column and this volume has generally to be reduced by evaporation. Purging the extracts with nitrogen or helium in precalibrated tubes [66], Kuderna-Danish evaporation [66] and rotary evaporation [72] are the methods of choice. Losses may occur in the preconcentration of the derivatized species especially for the more volatile Me_3Pb^+ species. Better recoveries are obtained when the solution of the organolead chelates, which are less volatile than TAL species, is preconcentrated prior to derivatization. However, a minimum volume of 250 μ l is required for easy handling during the derivatization step and removal of the unreacted Grignard reagent, inducing a dilution factor of 1:250 for capillary GC analysis. A preconcentration factor of 1:40 000 was reported from 10 l water samples [50].

Large volume injection techniques which have recently contributed to the elimination of the need for off-line enrichment will be discussed in section 14.3.1.

14.2.4 Derivatization of ionic organolead compounds

The non-polar character, volatility and thermal stability of tetraalkyllead species make them suitable for gas chromatographic separation. This is not the case, however, for both ionic organolead compounds and their chelates which must be converted into forms accessible to gas chromatography by means of derivatization. The derivative chosen must preserve the structure of the lead-carbon bonds to ensure that the integrity of the species remains unaltered. Attempts to employ hydride generation failed apparently due to insufficient reproducibility, abundant interferences and instability of the organolead hydrides [74,75]. The most common derivatization procedures involve ethylation with sodium tetraethylborate ($NaBEt_4$) [77] and propylation [51,53,54,66], butylation [42,66,72] and phenylation [73] using the Grignard reaction. A careful optimization of hydride generation conditions, however, promises some viable methods in the near future [76].

Out of the species shown in Figure 1 only Me_3Pb^+ can be derivatized unambiguously by ethylation. $\text{Me}_2\text{Pb}^{2+}$ and Me_2EtPb^+ occurring in the environment would form the same species upon derivatization, i.e. $\text{Me}_2\text{Et}_2\text{Pb}$. Besides, the ethyllead compounds: $\text{Et}_2\text{Pb}^{2+}$ and Et_3Pb^+ produce the same signal after derivatization as inorganic lead which makes ethylation useless for these compounds. An interesting feature of ethylation is, however, that it may be performed directly in the aqueous phase using the relatively water stable NaBEt_4 [77].

Propylation and butylation using the Grignard reaction are apparently the best choice at present. Smaller molecular weight and larger volatility of propylated species make their gas chromatographic separation faster than that of butylated species with less column carryover problems associated with derivatized inorganic lead. In addition, the baseline is more stable and less Grignard reagent related artefacts occur which is essential in ultratrace analysis. Some problems were reported with incomplete derivatization of dialkyllead species by butylation [59,66] but applying a larger amount of the derivatizing agent in solvents such as octane or nonane instead of hexane apparently solved the problem [51,72]. Resolution problems may be expected if two derivatives have the same total number of carbon atoms. From this point of view butylation is preferable to propylation. In the latter case several pairs of products, mostly including mixed methylethyllead species, are poorly resolved whereas the only resolution problem after butylation seems to exist between $\text{Me}_2\text{Bu}_2\text{Pb}$ and Et_3BuPb . The use of high performance capillary columns alleviates the resolution problems and will be discussed in section 14.3.2.

The use of a phenylation procedure seems to be less convenient owing to the increased formation of artefacts that is probably due to the relatively large stability of the phenyl radical which promotes redistribution reactions [73].

The unreacted Grignard reagent must be destroyed prior to the injection onto a column of the derivatized extract which is obtained by shaking the organic phase with dilute sulphuric acid. The organic phase is finally dried over anhydrous Na_2SO_4 and injected onto a GC column. The injected extract must not contain substances such as salts which remain in the injector after analysing a series of samples.

14.3 Instrumental aspects of the determination of organolead compounds

14.3.1 Gas chromatography based hyphenated techniques

14.3.1.1 Sample introduction

Conventional packed column injection ports are used for the sample introduction on packed and megabore columns. In case of capillary columns, however, unless a special injection technique is used, the low maximum allowable sample volume which may be introduced on the column negatively affects the experimental detection limits as only a tiny fraction of the derivatized extract is finally processed in the hyphenated system.

Recent advances in the electronic pressure control and retention gap techniques have enabled injection of large volumes on a capillary column. However, in many cases the large throughput of solvent rapidly dirties the detector, which results in the degradation of the analytical performance of the system. An alternative approach involves the removal of the solvent *on-line* prior to the transfer of analytes onto the

column. A precolumn trap enrichment of the analytes on Tenax was proposed a decade ago for preconcentration of butylated organolead species but operating the trap in the *off-line* mode appeared to be inconvenient [31].

Recently a commercial injection system was optimized for *on-line* preconcentration of derivatized organolead species [53]. The introduction of the sample onto a GC column makes use of different volatilities of the solvent and the analytes. It consists of three consecutive processes taking place in the injection liner: sample injection, solvent venting and release of the analytes onto the column. The principle of operation is shown in Figure 5. The solution of organolead species in a volatile solvent is injected into a cool injection liner (0 - 10°C). Then the temperature is slowly raised to 20 - 25°C in order to increase the vapour pressure of the solvent. The temperature is maintained at this level for 1-5 min while a stream of helium passes through the liner sweeping the solvent off the column. The less volatile analytes are kept in the liner until the volume of the solvent is reduced to 1 µl or less. The purge valve then switches the stream of the carrier helium gas into the column while the temperature of the liner is raised to the effective injection temperature, allowing a prompt release of the analytes to be achieved. The optimization of the system involves the choice of the liner (and packing material if necessary), the volume of the injected sample, the temperature at which the solvent is removed and the flow rate of the helium purge gas. The design of the liner and the kind of its packing (if any) should allow for the effective separation of picogram amounts of the analytes from a ten orders of magnitude or more larger bulk of the solvent. In addition, the liner should enable instantaneous release of the retained analytes by an increase in temperature to avoid broadening and/or tailing of chromatographic signals. Up to 25 µl can be processed at a time [53]. Larger amounts can be handled by successive injections of 20 - 25 µl volumes at 1 min intervals to remove the solvent [54].

14.3.1.2 Separation of the analytes

Non-polar phases have been recommended in the literature for the separation of derivatized organolead species [12]. 3-10 % loadings of OV-101 on Chromosorb W have been most frequently used [41,42,50,66,72,78,79]. There is a strong trend, however, to replace them by open-tubular megabore [80] or capillary columns with polymethoxysilane coatings (DB-1, HP-1, RSL-150). Capillary columns have been used mostly in combination with MIPAES [81-87] and very seldom with GFAAS [88] or QFAAS [89]. Packed columns do not allow for effective resolution between $\text{Me}_2\text{Pb}^{2+}$ and Et_3Pb^+ when butylation is used as the derivatization technique. In the case of propylation mixed methylethyl species may interfere in the determination of Et_3Pb^+ due to the same number of carbon atoms but this problem has never been investigated. Figure 6 presents the resolution of butylated organolead compounds in gas chromatography on packed, megabore and capillary columns. The latter combined with large volume injection techniques are likely to replace the packed ones especially in connection with the recent interfaces with AAS, which allow for more sensitive measurements.

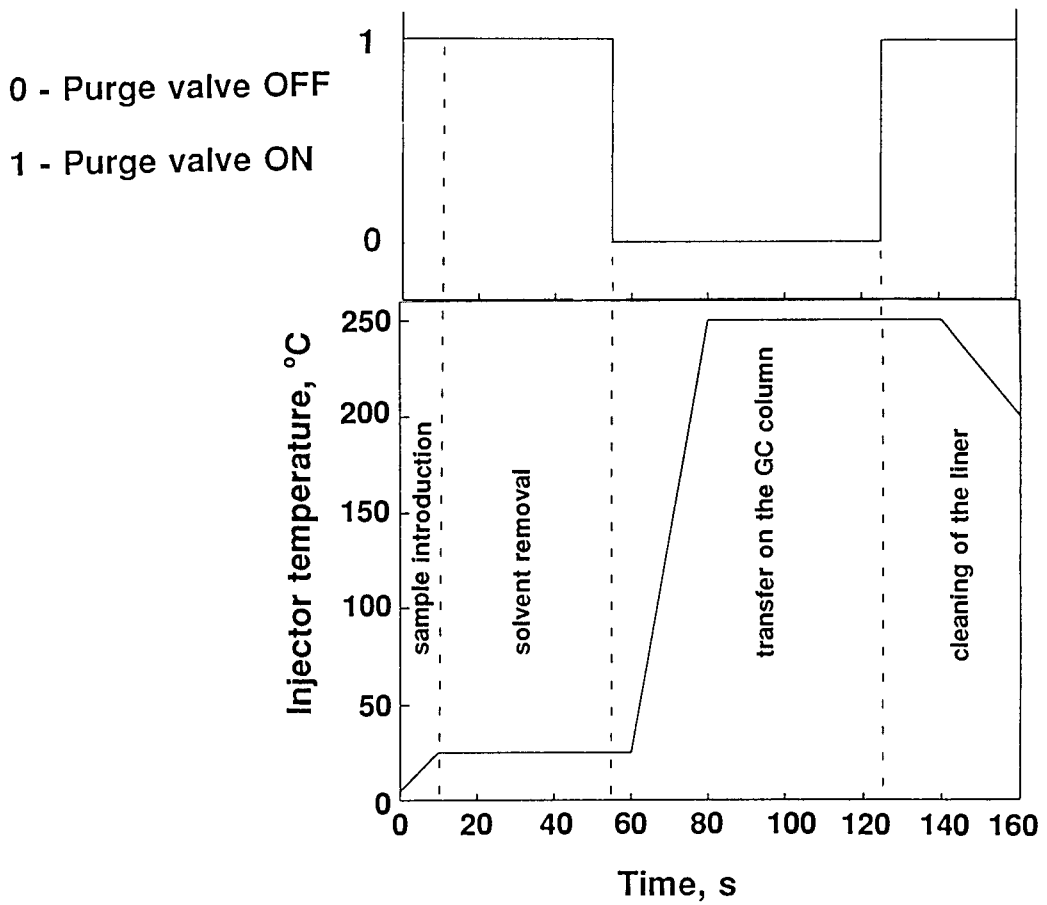
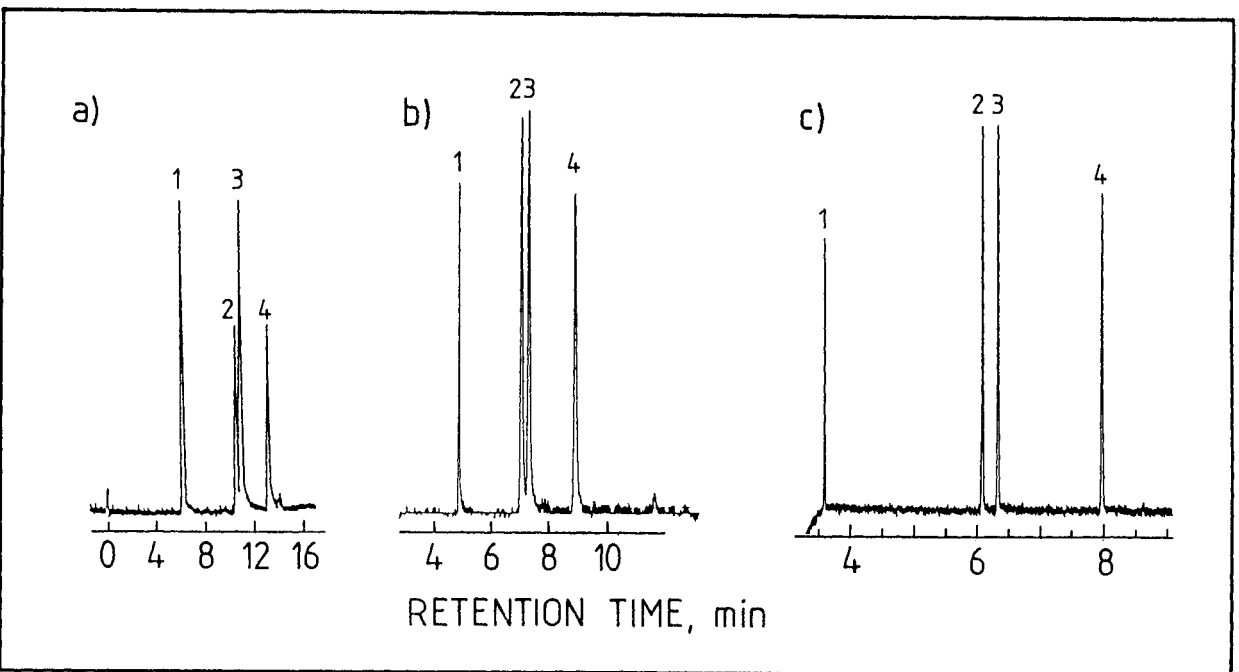


Figure 5: Principle of introduction of large extract volumes on a capillary column.

**Figure 6:**

Resolution in the separation of butylated organolead compounds on packed (A), megapor (B) and capillary (C) columns

The high resolution of capillary columns is essential for the analysis of complex environmental matrices and especially for biological samples. In addition, open-tubular columns may be operated at lower carrier gas flow rates than packed columns which improves sensitivity [90]. The inert nature of capillary column, makes them superior for the separation of organometallic species containing very polar bonds (metal-halogen) which may strongly interact with the packed column often resulting in the decomposition of the analyte and peak tailing. Other advantageous features of capillary columns include flexibility, ease of use and diversity of available coatings.

14.3.1.3 Transfer lines

Tetraalkyllead compounds decompose to some extent in heated metal or refractory oxide transfer lines. Suitable approaches include the use of a piece of deactivated fused silica or a section of the column as transfer line. The former solution allows easy changing of the column without resetting the interface.

14.3.2 Detection techniques

14.3.2.1. Atomic absorption spectrometry (AAS)

Quartz furnace AAS and graphite furnace AAS have grown in maturity during the past decade and are well recognised techniques for speciation analysis of organolead in several laboratories. The highest sensitivity is obtained using a silica furnace atomizer, heated either electrothermally or by an air-acetylene flame and placed in the path of the light-beam. Absolute detection limits below 10 pg were reported. The detector response depends on hydrogen and carrier gas flow rates, and dimensions of the atomization cell. Hydrogen is necessary to enhance the atomization, otherwise an appreciable portion of lead eluted from the GC column is not atomized and deposits on the wall of the silica tube.

Only few novelties regarding GC-AAS analysis for organolead appeared since the last review by Radojević [12]. The important ones have concerned the development of an interface for the megabore [80] and capillary column [89]. The adaptation of the designs used earlier in the packed column GC (PGC) - QF AAS coupling resulted in insufficient resolution (especially for $\text{Me}_2\text{Bu}_2\text{Pb}$ and Et_3BuPb) and peak tailing [78]. The reason for these problems was probably the enhanced decomposition of analytes outside the atomization zone of the furnace due to a smaller linear velocity through the GC-AAS interface when a capillary column was used in place of a packed one. The extension of the interface heating zone to the edge of the detector casing in order to enhance the temperature gradient along the furnace side arm allowed most of these problems to be solved. A better understanding of the role of hydrogen radicals in the atomization mechanism of lead inside the quartz furnace enabled further improvements. They included the addition of a heated quartz lined ceramic insert (acting as *in-situ* hydride generator) to the interface and in consequence transferring the analytes as hydrides into the upper tube of the quartz T-furnace [89].

For an optimized GC - QF AAS system a detection limit of 1.6 - 2.3 pg was obtained for lead which was a factor of 5 lower than ever reported before [67]. The megabore GC (MGC) - QF AAS interface recently developed [91] resulted in an improvement in sensitivity of a factor of 2.5 in comparison to the PGC - QFAAS interface [72]. The setup is described in detail in this book in chapter 15.

Despite recent progress in the coupling of a capillary column to a graphite furnace atomizer, GC - GFAAS remains inferior to GC - QF AAS both in terms of sensitivity and ease of operation. The detection limits were 8 pg (as Pb) for Me_4Pb and rapidly degraded for higher boiling species [65,88]. Problems associated with the long-term operation of a graphite furnace at high temperatures make the use GC - GFAAS inconvenient for routine analysis.

Although considerable improvements in the interface design were made, all GC - AAS systems developed so far have shown a signal discrimination with decreasing volatility of analytes.

14.3.2.2 Atomic emission spectrometry (AES)

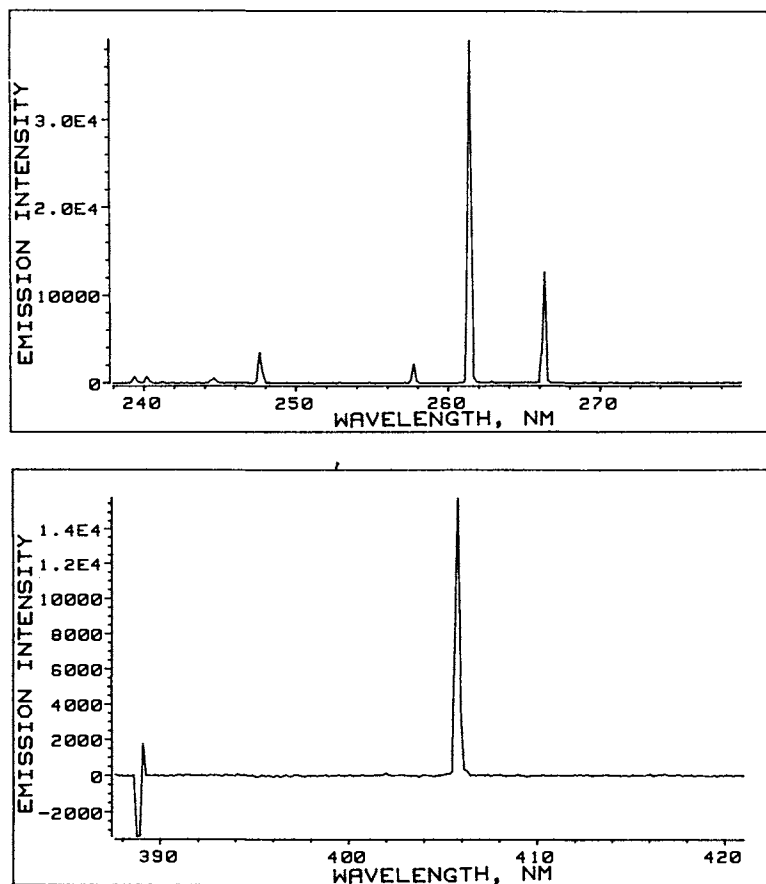
Plasmas are comparable to both a chemical combustion flame and an electrothermal atomizer with respect to the efficiency of the excitation of elements. The higher temperatures obtained in the plasma result in increased sensitivity and a large number of elements can be efficiently determined. Microwave induced plasmas are preferred to inductively coupled ones for atomization of the GC effluent due to lesser dilution of analytes by reagent gases in the plasma resulting in better detection characteristics [92-94]. Helium is preferred to argon as the plasma gas due to the higher energy provided which result in an easier excitation and consequently to a more sensitive detection especially of non-metals. However, it was only the introduction of a new type of cavity (TM_{010}) by Beenakker in 1976 that made it possible to achieve a stable operation at low power with helium at atmospheric pressure [95]. Along with an improvement in the detection limits by at least one order of magnitude for most elements, it offers an optimum precision and long term stability. The radiation emitted from the plasma is viewed axially and therefore neither formation of deposits on the wall of the discharge tube nor devitrification of the latter may result in changes in sensitivity.

For lead, detection limits usually quoted for laboratory-built instruments are in the sub-pg range, which is 1-2 orders of magnitude lower than those of AAS. The use of a computer controlled high performance monochromator with a diode array spectrophotometer with very efficient background correction allowed these values to be decreased to a level of 0.03-0.1 pg depending on the compound [51]. Operating conditions are very critical especially with respect to the helium makeup flow rate and the hydrogen (acting as scavenger) flow rate. For example, reducing the helium makeup flow by 50 % cuts the response by more than two orders of magnitude.

The 283.3 nm line was most often used for the measurement of lead emission with laboratory-built systems. In the commercial instruments the software controlling the data acquisition allows the measurement of lead emission at the 261.418 nm and 405.783 nm lines. The latter gives a response almost three times as high as the former.

The technique offers a unique opportunity for the confirmation of the elemental identity. Although the photodiode array spectrophotometer coupled to a monochromator provides a very good selectivity for lead for the analysis of real environmental extracts, hydrocarbons or other volatile organic compounds present at high concentrations may give rise to several interfering peaks in the chromatograms. The identification of each peak may be performed by comparing the emission spectrum registered at the peak apex (snapshot) with the pattern obtained for lead.

Such snapshots, taken in the vicinity of lead analytical lines (*i.e.* 261 and 406 nm) are shown in Figures 7 A and B, respectively. The pattern close to the 406 nm line which contains the lead line only is not considered to be the most reliable one. The spectrum in the range 240-280 nm should therefore be considered instead since lines at 247.638 nm, 257.726 nm, 261.418 nm and 266.317 nm may easily be identified as lead emission lines [96,97].



Figures 7A and 7B: Snapshots taken at the apex of an organolead peak in the vicinity of the 261 nm line (A) and the 406 nm line (B)

Another noteworthy feature of MIP-AES is the possibility to monitor the changes in emission intensity in the vicinity of the analyte line during the chromatographic run in order to indicate any possible spectral impurities. Peaks for the butylated $\text{Me}_2\text{Pb}^{2+}$ and Et_3Pb^+ species are shown in Figure 8, lying very close to each other.

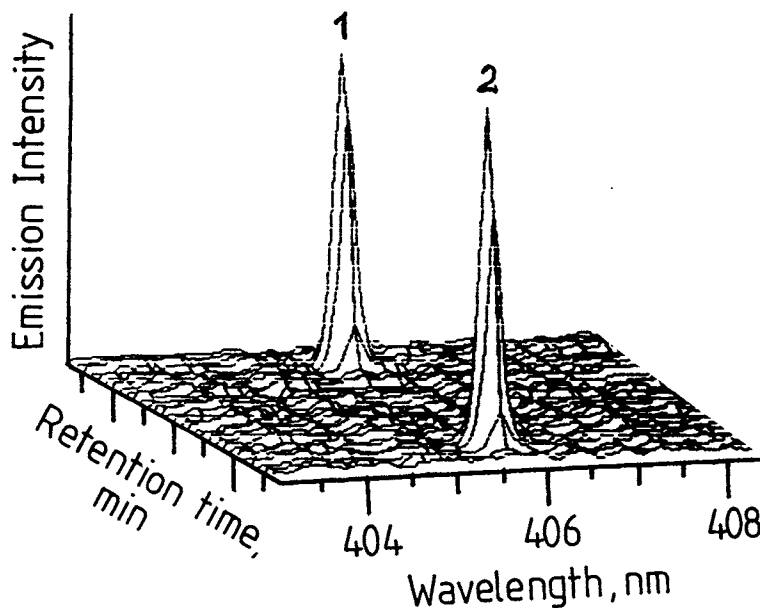


Figure 8: A three dimensional snapshot taken during the separation of butylated organolead compounds. 1- Et_3BuPb and 2- $\text{Me}_2\text{Bu}_2\text{Pb}$

The MIP is considered to be the cheapest analytical plasma but, nevertheless, the cost of a commercial instrument well exceeds 100,000 ECUs making it not readily available to most laboratories. Laboratory-made interfacing between GC and MIPAES is not so straightforward as in other hyphenated techniques due to the necessity of preventing the solvent vapours from reaching the plasma. Another disadvantage of the MIP compared with other plasma discharges is the limited thermal energy which results in difficulties with sample desolvation and atomization thus leading to low tolerance to the introduction of even a relatively small amount of sample. Therefore, prior to entering the detector the analytes must be well separated from the solvent.

The applications of other plasmas: alternating current plasma, capacitively coupled plasma to speciation analysis of organolead are scanty and the detection characteristics obtained do not match those offered by MIPAES [98,99]. Some unexplored potential seems to remain in DCPAES [86].

14.3.2.3 Mass spectrometry (MS)

Reports on the use of mass spectrometry (MS) for the detection of organolead in the GC effluent are rare despite the potential attractiveness of this technique. Electron impact ionization mass spectrometry (EI MS) in the single ion monitoring mode was reported to offer an absolute detection limit at the 1 pg level [52], which, together with the large popularity of this technique in organic chemistry, makes it a valuable tool for speciation analysis. Similar detection characteristics are offered by CGC - ICPMS; however, higher equipment and running costs and the necessity of a laboratory-made interface [100] makes this technique unaffordable for many laboratories. Sub-pg detection limits obtained with GC - MIPMS for organotin [101] foreshadow a large potential of GC with plasma mass spectrometric detection for the determination of lead.

14.3.3 Liquid chromatography (LC) based hyphenated techniques

LC based coupled techniques offer the possibility of avoiding the time consuming and interference prone classical derivatization step. Organoleads are separated as original species [45-47,102-108] or using ion-pairing reagents: ammonium tetramethylenedithiocarbamate [71,75] or sodium pentane sulphonate [48,109] using reversed phase chromatography (Nucleosil C₁₈) [47,48,103-105,109] ion-exchange chromatography (Partisil SCX-10) [46,106] or size exclusion chromatography [45]. The main limitation of LC for lead speciation analysis is the poor availability of sensitive, element selective detectors for the analysis of environmental and clinical samples. The major requirements for the coupling of LC to any atomic or mass spectrometric technique are the compatibility of flow rate and mobile phase with the detector, unless post-column on-line derivatization or fractional sample collection are used. The principal detectors used after the LC separation of organolead have included AAS and MS and the methods developed are summarized in Table 1.

14.3.3.1 LC-AAS coupling

Analysis of consecutive portions of the eluate by ETAAS is the oldest but the least elegant solution. It is based on the transfer of the aliquots from the effluent stream into the furnace tube at regular intervals, which is then heated in programmed steps, viz., drying, ashing and finally atomization. The effluent from the HPLC has to be stored in an autosampler or the system has to be operated in the stopped flow mode. Problems with the identification of the eluted analyte peaks are common. Loss of organolead due to the low temperature volatilization prior to atomization is avoided by adding oxides of transition metals, e.g. Cr₂O₇²⁻. However, the procedure is time-consuming and the resolution is poor.

A direct interfacing of LC with an atomic absorption spectrometer requires that the column eluate be introduced into the optical beam of the spectrometer in a continuous and pulseless mode. It can be done *via* a continuous thermospray interface or a nebulization chamber. A thermospray-microatomiser assembly was the most promising system and gave sub-ng sensitivities for the analysis of water [71]. Microbore columns and efficient nebulizers seem to offer a promising approach.

Table 1: Applications of liquid chromatography to speciation analysis for lead

Analyte	Column (mobile phase)	Post-column reaction	Detection	Detection limit	Sample [Ref]
TAL	C ₁₈ (98% MeOH)		GF AAS	n.g.	gasoline [102]
IAL	C ₁₈ (0.06% NH ₄ TMDTC in 80% MeOH)	ethylation with NaBEt ₃	QF AAS	0.10-0.15 ng	no real samples [75]
IAL	C ₁₈ (0.1M acetate buffer in 20% MeOH)	reduction of R ₃ Pb ⁺ to R ₂ Pb ²⁺ and complexing by PAR	photometric	15-20 ng l ⁻¹	urine [47], rain, snow, surface water [104]
TAL	C ₁₈ (50-70% EtOH)*		ICP AES	2-11 ng	gasoline [105]
TAL	Partisil SCX-10 (BuOH:EtOH: H ₂ O 15:35:50)		ICP AES	33-100 pg s ⁻¹	no real samples [106]
Protein bounded lead	size exclusion column (0.1 M Tris/HCl buffer, pH 7.2)		ICP MS	0.04 µg l ⁻¹	human and rat blood [45]
Me ₃ Pb ⁺	Partisil SCX-10 (0.167 M ammonium citrate in 70% MeOH)		ET AAS	n.g.	blood and soft mammalian tissue [46]
IAL	C ₁₈ (0.06% NH ₄ TMDTC in 75% MeOH)		QF AAS	1.0-1.8 ng	water, soil, sediment [71]
TAL	C ₁₈ (70% acetonitrile)		flame AAS	10 ng	gasoline [107]
TAL	Hitachi Gel 3010 (MeOH)		GF AAS	n.g.	gasoline, exhaust gases [108]
TriAL	C ₁₈ (5 mM ammonium pentane-sulfonate in 20% acetonitrile)		ICP MS	0.2 pg	urine (spiked) [48]
TAL, triAL	C ₁₈ (8 mM sodium pentane-sulfonate in 30% MeOH; 30% MeOH; 90% MeOH)		ICP MS	0.14-3.9 ng	gasoline [109]

* authors presented a wide choice of acceptable mobile phases; n.g - not given

Another possibility relies on a post-column reaction converting the analytes into a volatile form prior to their transport to the detector. Desolvation can thus be avoided and a better sensitivity is obtained. This is a common way of interfacing HPLC to AAS for tin and arsenic speciation analysis but for organolead compounds this procedure has been hampered by the lack of a suitable reaction. Trialkyllead compounds were found to be volatilized as hydrides from acid solution but the efficiency of volatilization was not high [75]. No hydrides were, however, obtained for dialkyllead compounds. Ethylation using NaBEt_4 was proposed as an alternative [75]. However, condensation of species with higher boiling point and blank value problems due to inorganic lead converted to Et_4Pb (similarly to Et_3Pb^+ and $\text{Et}_2\text{Pb}^{2+}$) are likely to be limiting factors especially at low concentration levels. Continuous post-column ethylation is compatible with water rich mobile phases permitting a variety of column separatory modes to be employed. No application was reported for real samples analysis for which serious problems are likely to appear especially in the case of polluted waters, due to the presence of high amounts of organic matter.

14.3.3.2 LC-MS coupling

Plasma detection for LC separations usually suffers from the inability of the plasma to tolerate large amounts of a volatile organic solvent (methanol) necessary for the elution of the analyte from the column. Also, during nebulization much of the sample is lost and the dead volume of the spray chamber is responsible for band broadening. A remedy may be a microconcentric pneumatic nebulizer placed inside the ICP torch (the direct injection nebulizer) which has a low dead volume ($<2\ \mu\text{l}$) and provides absolute detection limits superior by 1-2 orders of magnitude to those obtained with conventional nebulizers. It enables the use of microscale LC columns and liquid flow rates ($30\text{--}100\ \mu\text{l}\cdot\text{min}^{-1}$) that are low enough for all the column effluent to be introduced into the plasma [48,109]. Absolute detection limits of $0.2\ \text{pg}$ were reported [48] for organolead determinations using size-exclusion chromatography ICP MS.

14.3.4 Electrochemical techniques

The electrochemical techniques used for speciation analysis of alkyllead compounds include differential-pulse anodic stripping voltammetry (DPASV) at a mercury film electrode [110], a hanging mercury drop electrode [10,11] or at a mercury-plated glassy carbon cathode [111]. Interference from inorganic lead (Pb^{2+}) in the sample is prevented by its chelation with EDTA [112] or removal by co-precipitation with barium sulphate [112]. Amperometric and pulse-amperometric techniques were recently proposed for the detection of tetraalkyllead species after their HPLC separation [113]. The applications of electrochemical techniques to real sample analysis are rather rare and are summarized in Table 2.

Table 2: Electrochemical methods used for speciation analysis of lead

Analyte	Method	Detection limit	Sample [Ref.]
IAL	DPASV	n. g.	pigeons tissue (brain, kidney) [114]
IAL	DPASV	10^{-10} mol l ⁻¹ (dialkyllead) 2×10^{-10} mol l ⁻¹ (total alkyllead)	estuarine water [111]
IAL	DPASV	$0.02 \mu\text{g g}^{-1}$	birds tissue [115]
IAL	voltammetry	2×10^{-10} mol l ⁻¹	seawater [112]
IAL	DPASV	1.25 ng	seawater [10]
TAL	HPLC-amperometry HPLC-pulse amperometry	$1.5\text{-}1.7 \mu\text{mol l}^{-1}$	no real samples [113]
IAL	DPASV	n. g.	fish [116]
TAL, IAL	DPASV	$0.05 - 0.1 \mu\text{g l}^{-1}$	no real samples [11]

14.3.5 *Summary of characteristics of hyphenated techniques used for organolead speciation analysis*

Figure 5 shows a comparison of the absolute detection limits in various hyphenated techniques proposed for lead speciation analysis. Gas chromatography has been long preferred to HPLC separation due to its larger resolving power, easier identification of unknown compounds, a simpler carrier background and availability of more sensitive detectors; however, recent advances in HPLC - ICPMS coupling have made the latter technique equally suitable for ultratrace analysis [48]. Electrochemical techniques do not match spectrometric ones only in terms of sensitivity but are prone to interference and require a complex sample preparation.

Silica furnace electrothermal AAS is the most widely used detection technique in GC of organolead compounds. It offers relatively low detection limits (1-10 pg) and can be readily assembled in the analytical laboratory. Better characteristics can be obtained using microwave induced plasma atomic emission spectrometry or inductively coupled plasma mass spectrometry; these, however, are far more expensive. Absolute detection limits of 0.1 pg or lower reached by these techniques make them methods of choice for the future.

Molecular ions MS techniques offer an average detection limit of 1 pg but very recent improvements in EI bench top mass analyzers are likely to decrease this by a factor of 10 soon. The relatively low cost and versatility of these detectors makes it an interesting alternative for the future.

14.4 Analysis of environmental materials

A general layout of the sample preparation sequence is shown in Figure 4. For LC the sample preparation is restricted to chelation, liquid-liquid extraction and sometimes evaporation. Some specific considerations are discussed below. In the case of air, sample preparation is usually combined with sampling and is discussed in Chapter 17.2.1.1.

14.4.1 *Analysis of water samples*

Tetraalkyllead compounds may be quantitatively extracted into hexane [30] but have never been detected in real samples. Ionic organolead (IAL) species are usually extracted as diethyldithiocarbamates [10,42,66,72] into an organic solvent and then propylated [51,53,66] or butylated [31,42,66,72] with a Grignard reagent. The amount of the sample taken for the analysis is dependent on the organolead concentration and the detection technique used. For the analysis of less polluted samples by AAS large volumes (up to 10 l) were analyzed [50] while a 15 - 100 ml sample is sufficient for GC-AED [51,53,54].

An enrichment step is needed unless a large volume injection technique [53] is used. Evaporation of the extract before derivatization is very effective for preconcentration of IAL at the expense, however, of the determination of TAL in the same run. Conditions for the enrichment of tetraalkyllead species by evaporation are very critical and losses of more volatile species can hardly be avoided. Recoveries are usually quantitative although some authors observed lower extraction yields for dialkyllead species. Also for unfiltered samples adsorption losses on particulate matter may account for up to 20% of the tetraalkyllead present [30,59].

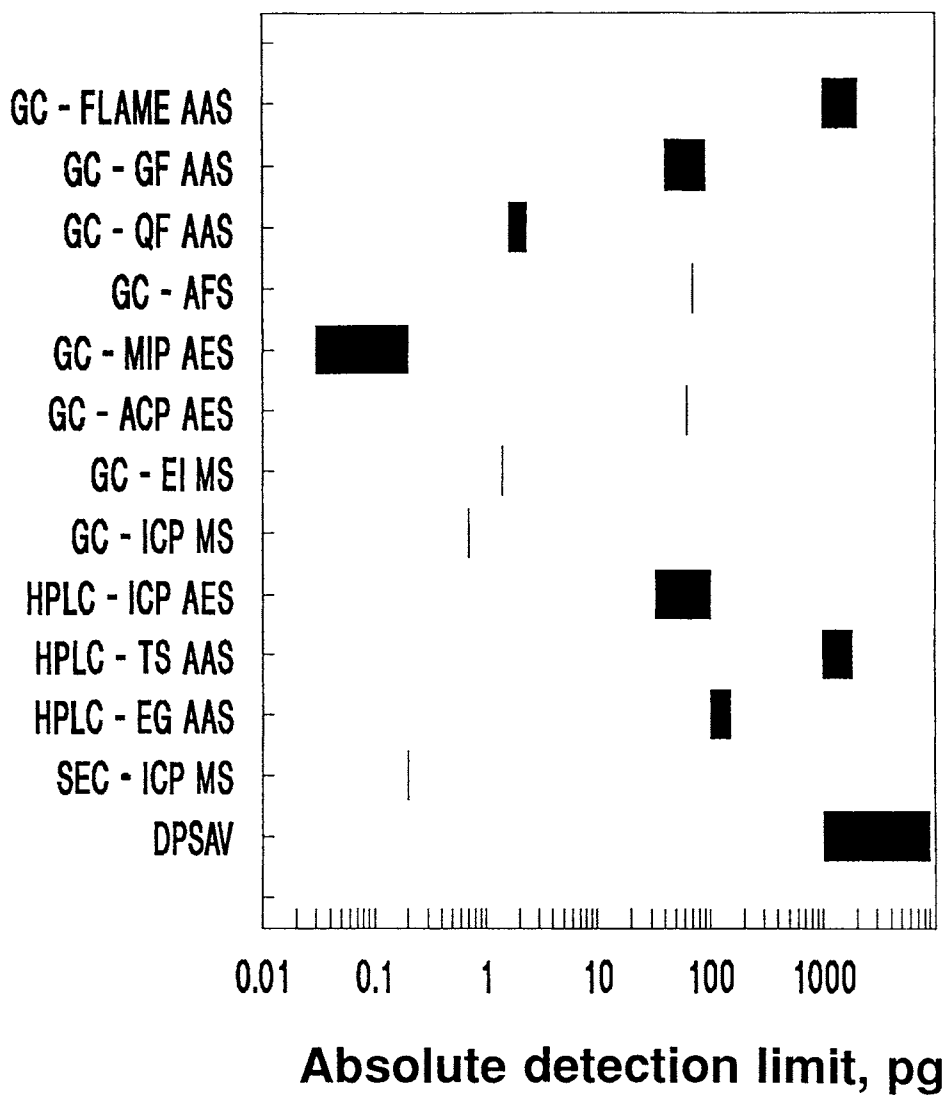


Figure 9: Comparison of absolute detection limits attained by hyphenated techniques in speciation analysis of organolead compounds
 EI: electron ionization
 TS: thermospray ionization
 EG: ethylide generation
 SEC: size exclusion chromatography

The principal procedures for organolead speciation analysis of water are summarized in Table 3.

Analyte	Separation/preconcentration	Derivatization	Analytical method	Experimental detection limit, ng l ⁻¹	Application
IAL	DDTC extraction (pentane), rotary evaporation, dissolution in nonane	butylation	GC - QF AAS	1.25 - 2.5 [72]	potable [50], rain [72,118], river and lake [118] water, snow [118]
IAL, TAL	DDTC extraction (benzene)	butylation	GC - QF AAS	8	river water [117]
IAL	none	none	DPSAV	1.25 [10] 10 ⁻¹⁰ mol l ⁻¹ (R ₂ Pb ²⁺) [111]	seawater [10], estuarine water [111]
IAL	DMDTC	none	HPLC - QF AAS	1.0 - 1.8 ng	sediment, water [71]
IAL	separation of Pb ²⁺ by co-precipitation with BaSO ₄	none	Voltammetry	2 · 10 ⁻¹⁰ mol l ⁻¹	seawater [112]
IAL	sorption on silica gel	none	HPLC - photometry	15 - 20 ng l ⁻¹	rain, snow, surface water [104]
IAL, TAL	DDTC extraction (hexane)	propylation [20,28,49,51,53], butylation [27,28,30,51,58]	GC - QF AAS [20,27,28,30,49,58] GC - MIP AES [51,53]	2.8 - 50 [58] 0.1 - 1.0 [20] 0.1 - 2 [51,53]	drainage [30], surface [49], tap [51,53], potable [49] and rain [20,27,28,51,58] water, polar snow [53]

IAL - Me₂Pb²⁺, Et₂Pb²⁺, Me₃Pb⁺, Et₃Pb⁺, MeEtPb²⁺, Me₂EtPb⁺, MeEt₂Pb⁺

TAL - Me_nEt_{4-n}Pb; DMDTC - dimethyldithiocarbamate

Table 3: Speciation analysis for organolead in water.

14.4.2 *Sediments, soil and dust*

Any acid digestion of the sample prior to the determination of organolead would inevitably lead to the destruction of the analytes. However, since alkyllead compounds are apparently not involved in mineralogical processes, the total decomposition of the matrix is not necessary. Hence, the methods for the extraction of organolead from sediments, soils and dust matrices are exclusively based on leaching and solvent extraction.

In the early works, organolead in sediments was determined as hexane extractable lead or volatile lead. These data must be treated carefully since the hexane extractable lead fraction may include species other than alkyllead and differs in the presence of complexing agents whereas the volatile lead fraction depends on the volatilization conditions.

Solvent extraction is the method of choice for tetraalkyllead compounds and, in the presence of a complexing agent, for ionic organolead species. Hexane is usually the solvent of choice due to its virtual insolubility in water, low boiling point and low viscosity although some authors preferred benzene [42,117].

For the extraction of IAL compounds a complexing agent, usually DDTC, is mandatory. The sediment is usually agitated in an ultrasonic bath for periods from 15 s to 2 h. Although quantitative recoveries were observed for 15 s agitation it seems unlikely that such short sonication times could be used for more complex samples. EDTA is often added as an aid to disperse the sediment in a suspension and to produce a cleaner boundary between the aqueous and organic phase thus facilitating the phase separation. Sodium benzoate was reported to increase the recoveries of the IAL species [42]. Glass beads are sometimes added during extraction to increase the sediment specific site surface in order to prevent the fluffy sodium benzoate from forming lumps at the surface. Extracts may contain suspended particles stabilized by the solvent [62] and centrifugation is, therefore, recommended especially when the extracts are further concentrated. Solvent loss in sediment extraction is not a serious problem.

Satisfactory yields are usually obtained for tetraalkyllead and trialkyllead species but not for dialkyllead. Low recoveries were observed in n-hexane extracts. Benzene may be an alternative solvent but its use involves the hazard of significant co-extraction of organic matrix components because of its relatively high polarity. The reasons for extraction losses include decomposition, irreversible adsorption or interactions of organolead compounds with components of the sediment. Some soils contain sites capable of binding alkylleads (especially dialkylleads) to form hexane-soluble non-polar complexes resistant to the action of dilute acid [75].

Co-extracted components in sediment extracts may reduce the column lifetime and affect the separation (especially HPLC) leading to peak tailing and a clean-up stage is then usually necessary. Post-derivatization clean-up using alumina columns is the method of choice. An alternative is the use of dithizone for extraction and back extraction of the ionic compounds in the aqueous phase while organic matter remains in the organic phase.

Selected procedures for speciation analysis for organolead in sediment, soil and dust are summarized in Table 4.

Analyte	Separation/preconcentration	Derivatization	Analytical method	Experimental detection limit, ng g ⁻¹	Application [Ref.]
IAL	DDTC extraction (pentane), rotary evaporation, dissolution in nonane	butylation	GC - QF AAS		soil [50], aerosol [25]
IAL, TAL	DDTC extraction (benzene), rotary evaporation	butylation	GC - QF AAS	15 (wet weight)	river sediments [42,117]
IAL	DDTC, DMTC, APDC extraction (hexane), preconcentration under stream of N ₂	butylation, phenylation	GC - QF AAS		soil, street dust [73]
IAL, TAL	DDTC extraction (hexane)	butylation [58,61], propylation [20,61]	GC - QF AAS [20,58,61] GC - flame AAS [58,61]	0.6-10 (dust soil, sediments) [58], 1.3-35 (aerosol) [58]	street and road dust, soil, sediments [42,58,61], aerosol [20,58], deposits [20]
IAL	DMDTC extraction (hexane)	none	HPLC - QF AAS	1.0 - 1.8 ng	soil, sediment [71]

IAL - Me₂Pb²⁺, Et₂Pb²⁺, Me₃Pb⁺, Et₃Pb⁺, MeEtPb²⁺, Me₂EtPb⁺, MeEt₂Pb⁺
TAL - Me₄Et_nPb

Table 4: Speciation analysis for organolead in dust, sediments, soil and aerosol.

14.4.3 *Biological tissues*

Earlier research used leaching procedures to release organolead compounds from tissue homogenate [42]. Inorganic lead in an iodide/chloride solution was added as an aid to release the organolead from the tissue. Sodium benzoate was sometimes added to improve the extraction of dialkyllead while EDTA was used to complex inorganic lead, to disperse the fish tissue homogenate and to facilitate the phase separation. However, as organolead compounds may be incorporated in tissues of a living organism, solubilization or decomposition of biological materials prior to separation of the analytes is necessary. A suitable digestion procedure should allow for the complete destruction of the matrix while organolead species remain unchanged. Two principal approaches have so far been proposed. One involves digestion of the sample with tetramethylammonium hydroxide (TMAH) [35,42,117] while the other is based on enzymatic hydrolysis with a mixture of lipase and protease [67,119-122].

Solvent loss is a serious problem in fish extraction as part of the solvent is homogenized with proteins and fat. Clean-up is necessary because of high protein and lipid contents which might clog the GC column. An alternative is the separation of the organic matrix by back extraction of the ionic organolead from the dithizone extracts into dilute nitric acid [116].

Procedures used for the determination of organolead compounds in biological materials are summarized in Table 5.

14.5 Accuracy

Quality assurance principles are discussed in Chapter 1 of this book. Possible errors likely to occur during sampling and storage are discussed in section 2 of this Chapter. Here some potential errors arising during sample handling and the determination itself are highlighted.

14.5.1 *Contamination Risk*

Contamination risks are generally not serious in the laboratory since organolead compounds usually occur in samples at high concentrations levels in comparison to the atmospheric background. When ultratrace amounts are determined, however, any work to be performed with concentrated calibrants in the same room is strongly discouraged; otherwise persistent blanks may remain for several days. Potential sources of contamination include the glassware and the electrodes used for pH measurements. Cleaning the glassware in hot concentrated HNO_3 has proved to be efficient even after use of the vials for the storage of concentrated organolead calibrants [54]. For the electrode it is recommended not to immerse it into solutions containing high levels of organolead. Reagent impurities are removed by pre-extraction. A limiting factor is the purity of Grignard reagents which are not purifiable due to their low stability. PrMgCl was found to be the best choice in this respect [53]. Airborne contamination starts to affect the analysis at the levels below 0.1 pg.g^{-1} . Analytical procedure blanks of $20\text{--}30 \text{ fg.g}^{-1}$ are common unless samples are not handled on a clean bench [54]. While the efficiency of the HEPA filters used in clean rooms to remove adsorbed lead particles and organolead is beyond doubt, the degree of removal of the gas phase TAL is unknown.

Table 5: Speciation analysis for organolead in biological materials.

Analyte	Sample preparation	Separation/ preconcentration	Derivatiza- tion	Analytical method	Experimental detection limit	Application [Ref.]
IAL, TAL	homogenization, TMAH* hydrolysis (60 °C; 1-2 h)	DDTC extraction (benzene)	butylation	GC - QF AAS	8 ng g ⁻¹ (wet weight)	fish, clams, macrophytes [42,117]
Me ₄ Pb	haemolysis by freezing (-20 °C; 24 h)	extraction (<i>n</i> -heptane)		GC - GF AAS	10 ng ml ⁻¹	blood [44]
Me ₃ Pb ⁺	haemolysis by freezing	DDTC extraction (pentane)	butylation	GC - GF AAS	3 ng ml ⁻¹	blood [65]
TAL, IAL	homogenization, enzymatic (lipase VII, protease XIV) hydrolysis (37 °C; 24 - 48 h)	dithizone extraction (hexane)	butylation	GC - QF AAS		snails[119], birds organs and eggs [43,69,120] fish, shrimp, scallop [67]
IAL	pulverization in liquid nitrogen by crushing in the mortar, TMAH hydrolysis (room temperature; 2 h)	DDTC extraction (pentane), rotary evaporation, dissolution in nonane	butylation	GC - QF AAS		grass, tree and shrub leaves [35]
IAL	none	Sorption on silica gel	None	HPLC - Photometry	0.15 - 0.2 ng ml ⁻¹	urine [47]
IAL	homogenization	extraction with toluene, reextraction of IAL into dil. HNO ₃	None	DPSAV	n.g.	pigeons tissue [114]
total IAL	homogenization	extraction with toluene, reextraction of IAL into dil. HNO ₃	None	DPSAV	0.02 ng g ⁻¹	bird tissue [115]
R ₃ Pb ⁺	none	none	None	HPLC - ICP MS	0.2 pg	urine (spiked) [48]
Me ₃ Pb ⁺	Homogenation	Leaching with 3 M HCl for 18 h, extraction into chloroform - ethylacetate	None	HPLC - ETAAS	n.g.	blood and soft mammalian tissue [46]
Protein bounded lead	Hemolysis by freezing, sepn. of the hemolyzate from cell membrane fractions by centrifugation	none	None	HPLC - ICP MS	0.04 µg l ⁻¹	human and rat blood [45]

*TMAH - tetramethylammonium hydroxide

IAL - Me₃Pb²⁺, Et₃Pb²⁺, Me₂Pb⁺, Et₂Pb⁺, MeEtPb²⁺, Me₂EtPb⁺, MeEt₂Pb⁺TAL - Me₄Et₄Pb

14.5.2 Losses

Because of their high volatility tetraalkyllead compounds may be volatilized from samples to a degree dependent on their vapour pressure. Hence, samples should be stored frozen if analysis cannot be done on the day of sampling. Adsorption of tetraalkyllead compounds onto the walls of sampling bottles is likely to cause losses of more than 90 % within a few hours. Therefore, it is recommended to carry out the extraction of aqueous samples into n-hexane inside the sampling bottles immediately after sampling. No similar wall adsorption problems were noted for ionic alkyllead compounds.

14.5.3 Calibrants

Only few organolead compounds: Me_3PbCl , Et_3PbCl , Me_4Pb and Et_4Pb are commercially available at present to be used as calibrants. The syntheses of Me_2PbCl_2 and Et_2PbCl_2 were described in the literature [42,122,123]. The use of mixed methylethyl ionic calibrants has not been reported so far. These compounds were identified in some samples by prediction of retention times of the derivatized species using the Kovat's retention index [122] or on the basis of retention times of tetraalkyllead calibrants [123,124]. Actual retention times of the mixed compounds can be confirmed using transalkylation mixtures. Quantification of the mixed compounds is made by interpolation using the available calibrants. Discrimination of the response with the boiling point of the analyte must be taken into account.

Aqueous TAL calibrants should be prepared in ultrapure water just before use. Concentrated solutions of trialkyllead and dialkyllead can be stored in the dark for months without major deterioration. Working solutions should be prepared daily as dilute solutions demonstrate a slow degradation, and after prolonged storage, redistribution reactions may give rise to the formation of mixed compounds if methyl- and ethyllead species are kept together. The rate of decomposition is increased by solar radiation and depends on the water quality, being 20 - 50 times faster in distilled than in deionized water [61]. Since IAL salts decompose with time, quantification is often done using derivatized calibrants.

The preparation of propylated and butylated di- and trialkyllead calibrants is described elsewhere [51,72]. The calibrants are quantified by converting the organolead to inorganic lead with a mixture of concentrated HNO_3 and HClO_4 and determining lead by flame AAS. The purity of the calibrants is controlled by GC-AAS or GC-AED by checking on the presence of other organolead peaks.

Calibration is a serious problem in the analysis of air for organolead. R_4Pb calibrants were generated using a diffusion tube vapour generator [58] while gaseous R_3Pb^+ were generated from the chloride salts through a Millipore membrane [18].

14.5.4 Validation of the Results

In more than 90 % of the studies published up to now, organolead was determined by GC-AAS after chelate extraction followed by the Grignard derivatization. Neither the use of standard addition nor the reliance on synthetic calibrants can be regarded as the ultimate proof of accuracy as they do not always account for interactions between the analyte and the matrix. There is an urgent need for independent sample preparation procedures and comparison studies to throw more light on the accuracy of the published methods.

With regard to sample preparation for gas chromatography, hydride generation (restricted so far to trialkyl species) [75], ethylation (restricted to methyllead compounds) [77] and *in situ* butylation may offer alternative approaches to Grignard derivatization. Recent developments in HPLC and wider availability of ICP-MS are expected to result in alternative methodologies. Producing reliable calibrants is of paramount importance.

The first interlaboratory comparison study revealed relatively few calibration problems at a relatively high concentration levels (4 and 40 $\mu\text{g.l}^{-1}$ respectively) [125]. The techniques tested were mainly based on complexation (e.g. with EDTA), Grignard derivatization and GC or HPLC separation; the detection techniques were MIPAES, ETAAS, DPASV, MS, ICPMS and UV/visible spectrometry [125].

14.6 Conclusions

GC-AAS has grown in maturity as a technique for speciation analysis of organolead but unless capillary columns and efficient injection techniques become more popular it is expected to be gradually replaced by much more sensitive GC-AES or GC-MS.

Sample preparation remains an Achilles heel of the procedures used for speciation analysis for organolead. There is a trend to eliminate tedious off-line preconcentration by automatic pre-trap injection concentrating systems and more efficient extraction (solid-phase) techniques. Alternatives to Grignard derivatization are still not seen on the horizon although some progress has been made with *in-situ* derivatization using hydride generation and ethylation.

A wider availability of ICPMS is likely to contribute to a rapid development of HPLC as a separation technique providing an opportunity for independent analyses. The production reliable calibrants is an urgent matter as well as interlaboratory comparisons and production of reference materials certified for lead species to cast more light on the analytical accuracy.

The development of methodologies for ultratrace speciation of organolead is driven by the need to obtain in-depth profiles of the variations of organolead atmospheric concentrations over Antarctica and Greenland during the last century by analyzing deep snow records. This is expected to allow a reconstitution in detail of the evolution of the global lead pollution since the introduction of leaded gasoline. Another area of focused interest is the analysis of rainwater (control of atmospheric contamination), urban dust, food and tissue samples to evaluate the human intake and toxicity of organolead compounds.

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15.

Speciation analysis of organotin by GC-AAS and GC-AES after extraction and derivatization

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The widespread use of organotin compounds in agriculture, plastics industry and especially in the production of antifouling paints and the final release of these compounds into the environment have led to an increasing concern about their persistence and toxicity [1]. Unlike inorganic tin, organotin may occur at toxic levels in aquatic and sedimentary environments. The partition of organotin between these compartments, as well as its bioavailability and toxicity depend critically on the chemical form in which the species are actually present. This is why total tin determinations are not able to provide reliable information on the hazards of the release of this element into the aquatic environment making speciation analysis of utmost importance.

Many analytical methods for the determination of mono-, di- and some trisubstituted (methyl, butyl, phenyl and octyl) organotin compounds have been developed [2,3]. The most popular are hyphenated techniques based on the separation of organotin species by liquid or gas chromatography followed by on-line atomic absorption, emission or mass spectrometric detection. They were recently comprehensively reviewed [4-6]. The use of high performance liquid chromatography (HPLC) for the separation of organotins has recently been reviewed [7] and apparently the HPLC based techniques suffer from poor resolution and usually the lack of sensitivity. The approach involving gas chromatography (GC) is usually preferred due to the high resolving power of this technique accompanied by a relatively easy coupling to selective and sensitive detectors.

Atomic absorption spectrometry (AAS) is a well-established detection technique in speciation analysis. It is commonly available in an analytical laboratory and the interface is relatively simple and may be constructed using commercially available parts. Interfacing a gas chromatograph with an atomic spectrometer greatly simplifies the task of the atomizer since the analytes which enter it are already present in the gas phase. In theory, any kind of atomizer can be coupled to a gas chromatograph but

in practice several conditions need to be met in order to obtain good sensitivity and selectivity for specific analytical problems. Several different approaches have been made to exploit the quartz furnace (QF), heated electrothermally [8] or by flame [9], or the graphite furnace (GF) as the atomization device [10-13].

Irrespective of the atomizer used, atomic absorption spectrometry is a relatively unsensitive technique for the determination of tin due to the high atomization temperature and the possibility of the formation of refractory oxides. Plasmas providing higher atomization temperatures are much more advantageous. Gas chromatography coupled to an atomic emission spectrometer (GC - AES) was recently proposed as a technique for the speciation analysis of organotin compounds showing good selectivity and sensitivity [14-17]. Absolute detection limits were at the pg or sub-pg level.

In recent years a plethora of sample preparation methods and instrumental analytical set-ups have been developed for the speciation analysis of organotins in environmental matrices. These methods for the conversion of ionic alkyltins into gas chromatographable species include two basic categories: (1) those based on *in-situ* hydridization using sodium borohydride (NaBH_4) [17-20] or ethylation with NaBEt_4 [21-23], (2) and those based on the extraction of the ionic organotin compounds and derivatization using Grignard reagents: methyl- [24,25], ethyl- [15,26-31], propyl- [32,33], pentyl- [13,14,34-40], or hexylmagnesium [41,42] chlorides/bromides. The first group of methods mostly based on the formation of volatile organotin species in the aqueous phase and have become integrated with extraction. Some methods imply separation and the formation of the volatile species in the extract [19-23] or on the solid phase support [26-28,35,43,44].

The Grignard alkylation reaction proceeds quantitatively, leading to stable derivatives when it is carried out in a suitable solvent. Ethylation or pentylation are the usual choice as they allow a simultaneous speciation analysis of methyl-, propyl-, butyl- and phenyltin species. Pentylation leads to less volatile and toxic analytes than ethylation, which, on one hand, facilitates further preconcentration but, on the other hand, could account for condensation problems in the interface during gas chromatographic quartz furnace atomic absorption spectrometric (GC-QFAAS) analysis.

Generally, every procedure for speciation analysis consists of five successive steps: (1) separation of the analytes from the sample matrix; (2) formation of volatile derivatives, (3) preconcentration, (4) cleanup and (5) determination. There is a strong trend to reduce the number of steps by combining some of them. The major consideration has been shifting away from the determination itself toward the quantitative separation of the analytes from complex samples. A schematic layout of sample preparation for organotin speciation analysis is shown in Figure 1. Specific considerations with respect to water, sediment and biological samples are discussed in the paragraph on sample handling below.

This chapter gives a comprehensive review of different approaches to gas chromatography interfaced with atomic spectroscopy in speciation analysis for organotins in various matrices supported by original analytical results concerning the optimization and application of GC-AAS and GC-AED. Particular emphasis is given to factors affecting the accuracy of the procedures developed [13,14,37,45-47], *viz.*, preparation and quantification of standards and intermethod comparisons.

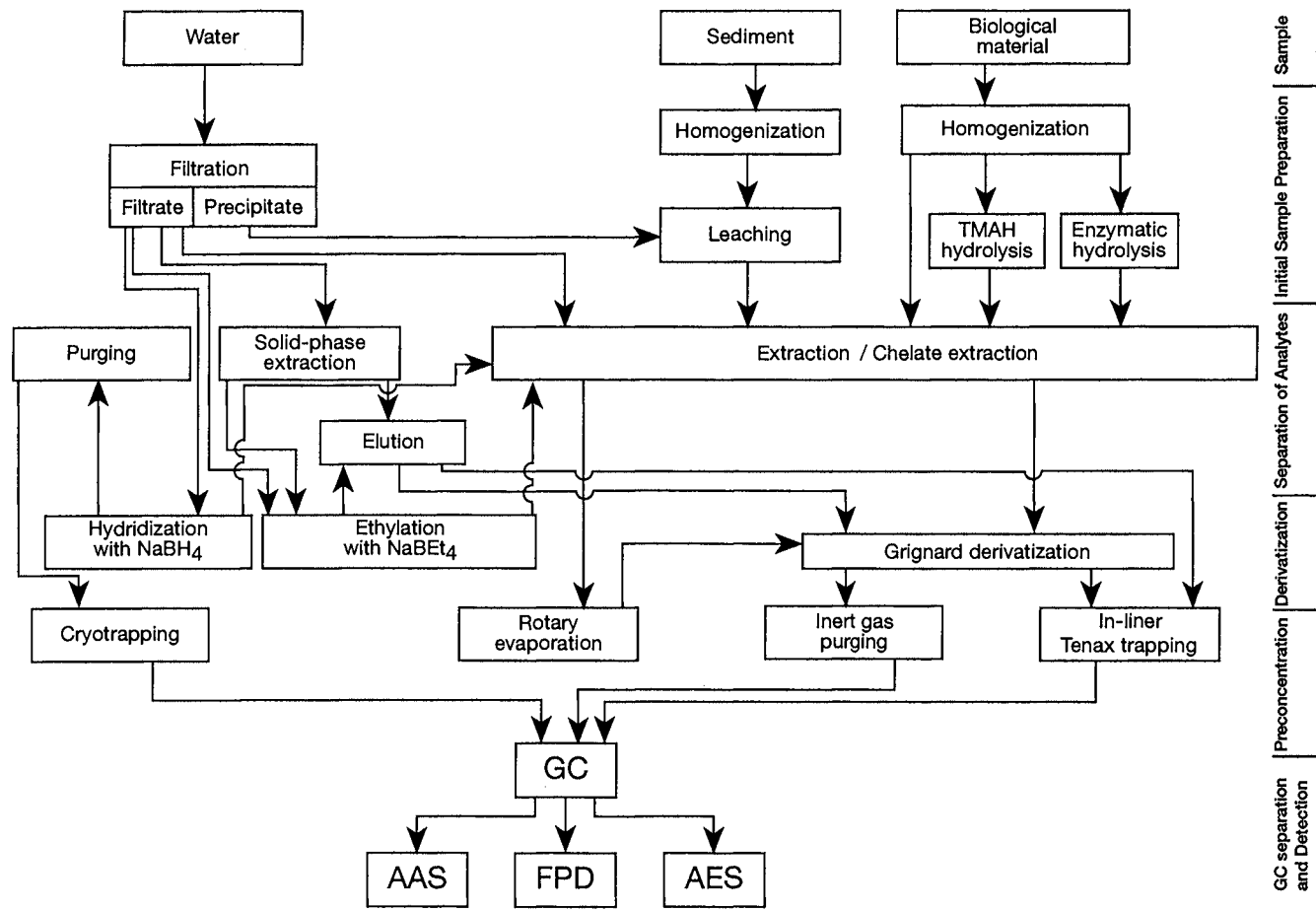


Figure 1: Schematic layout of sample preparation procedures for organotin speciation analysis

15.1 Instrumentation

15.1.1 Gas chromatography

A Varian Model 3700 gas chromatograph (Varian, Sunnyvale, CA) and an HP model 5980 Series II gas chromatograph (Hewlett-Packard, Avondale, PA) were used for the separation of the analytes before the detection by AAS or AES, respectively. Details for the columns used are shown in Table 1. Glass columns were silanized before packing with a 5 % solution of dimethyldichlorosilane in toluene.

Table 1: Details of the gas chromatographic columns used in this work.
 MAOT - Maximum allowable operating temperature.
 FSOT - Fused silica open tubular.
 df - Film thickness.
 N/m - Number of theoretical plates per metre.

Column	Packing or Coating	Dimensions	N/m
Packed (Chrompack)	3 % OV-101 on Chromosorb W HP (100- 120 Mesh) MAOT: 350 °C	1.8 m x 2 mm i.d. x 6 mm o.d.	2800
Megabore (RSL - 150)	Bonded FSOT 100 % polydimethylsiloxane MAOT: 330 °C	15 m x 0.53 mm i.d.; 1.2 µm (df)	2200
Capillary (HP - 1)	Crossed linked 100 % polydimethylsiloxane gum MAOT: 325 °C	25 m x 0.32 mm i.d.; 0.17 µm (df)	260 ⁿ

When a packed column was used, samples were injected directly onto the column. For the injection onto a megabore column a wide-bore on-column liner provided with a 1-m retention gap of 0.53 mm deactivated fused silica tubing (RSL, BIO-RAD, Eke, Belgium) was used. Injections onto a capillary column were made either using an HP split/splitless injector or a temperature programmed cool injection system (CIS) (Gerstel, Mülheim a.d. Ruhr, Germany). In the latter case a Tenax packed glass vaporisation tube (93 mm long x 1.25 mm i.d. x 2 mm o.d.) was used. Injections were made manually by means of appropriate Hamilton syringes except when 1 µl volumes were injected. In that case an HP model 7673A autosampler was used.

Data acquisition, processing and editing in the GC-AES measurements were made by means of an HP Model 5895A ChemStation. The data were stored either on a 80 MB hard disk or on tape cartridges. An HP model 9144 tape station was used. The graphic output was done using either a DeskJet printer or plotter. Various approaches to the acquisition of GC-AAS data were made throughout this study. GC-AAS chromatograms were recorded on a fast response chart recorder (Hitachi PE56) or on Spectra-Physics Model SP 4290 integrator operating in the peak height mode. This integrator was also connected to an IBM compatible computer using the Spectra-Physics LABNET[®] WINner[™] system. The SP WINner workstation Version 4.00 software was used to store or reprocess chromatograms and data.

15.1.2 Interface design

15.1.2.1 GC-AES

To connect the gas chromatograph and the atomic emission detector, a commercial interface (Hewlett - Packard) consisting of electrically heated and thermally insulated aluminum tubing was used. The final part (about 0.6 m) of the capillary column emerged from the GC oven through the Al-tubing and ended in a quartz discharge tube of a slightly larger diameter than the outer diameter of the column. The polyimide coating of the column was not removed by burning and scraping as recommended by the manufacturer. It was found that handling the column in this way occasionally created active adsorption sites inside the end-part of the column affecting the sensitivity and reproducibility of organotin determinations. The discharge tube was exchanged not more frequently than every three weeks.

15.1.2.2 GC - quartz furnace AAS

The packed column was connected to the quartz furnace of the atomic absorption spectrometer by means of a 1-m long nickel tubing (1/8 " o.d.). The latter was heated by applying a variable transformer controlled voltage between the tube ends which were well insulated from the rest of the instrument. The temperature was monitored with a thermocouple. The transfer line was connected on the GC-side to the base of the flame ionization detector (FID) *via* a stainless steel low hold-up union thus allowing the introduction of support gases (hydrogen and air) in a simple way. At the side of the AAS the transfer line was connected to the side arm of the quartz furnace using a Teflon union. This construction was earlier shown to be reliable for organolead determinations and is described and illustrated more in detail elsewhere [48].

The transfer line for the connection of the megabore column with the quartz furnace was made of a 2-m long section of deactivated fused silica tubing of the same diameter as the column. The column and the transfer line were connected by means of a push-and-fit capillary connector (0.53 to 0.53 mm, RSL, BIO-RAD). The transfer line was inserted into an insulated nickel tubing and heated as described above. The design of the interface at the atomic absorption spectrometer side is shown in Figure 2a. The connection of the heated nickel tubing of the transfer line to the side-arm of the silica furnace was made by means of a reducing union shown in detail in Figure 2b.

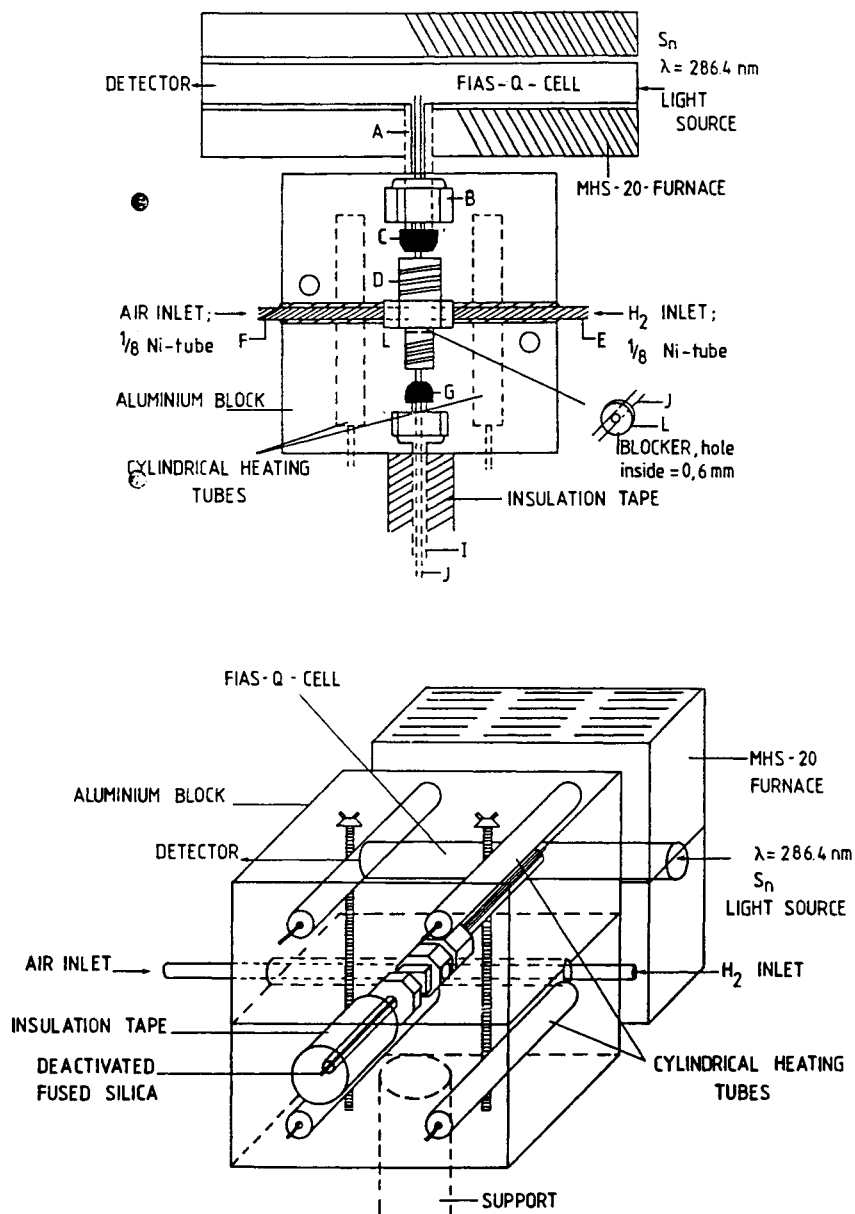


Figure 2a: The design of the GC-QFAAS interface at the atomic absorption spectrometer. Note I, II - different projections

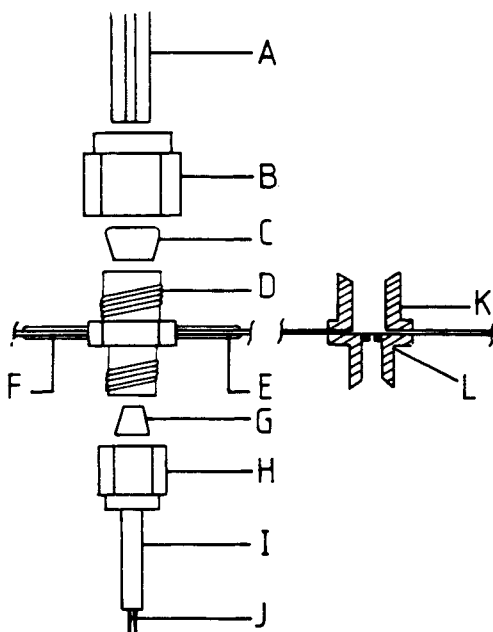


Figure 2b: Details of the reducing union connecting the transfer line to the side arm of the quartz furnace

It was made by modification of a conventional stainless steel reducing union (1/8" x 1/4") by drilling two holes in the middle part of it, perpendicularly to the axis of symmetry, in order to accept separate pieces of 1/8-inch nickel tubing. These holes served as inlet ports for the make-up gases to the quartz furnace and enabled the flow of the gases between the outer wall of the megabore tubing and the inner walls of the reducing union and the quartz furnace side-arm. The body of the reducing union was placed inside a 10-cm cubic aluminum block. The shape of the reducing union was milled in both parts of the Al block in order to make the union fit tightly inside the block during the assembly (lock-and-key connection). The block was made of two identical parts each containing two cylindrical heating elements. The heated Al-block ended just in front of the MHS-20 heating unit. The fused silica transfer line was pulled through the reducing union until the end of it was positioned a few millimeters inside the 1/4 inch part of the reducing union. The temperature of the Al-block was controlled by means of a thermocouple placed in the middle of the block. In the megabore column GC - QFAAS setup, the support gases: hydrogen and air were introduced into the quartz cell via the modified reducing union.

15.1.2.3 GC - graphite furnace AAS

Interfacing the transfer line with the heated graphite furnace atomizer was made in a way similar to that described by Radziuk *et al.* [12] and is shown in detail in Figure 3a. The end of the heated nickel tube was equipped with a tantalum connector of about 1.5 cm in length which was machined out of a tantalum rod of 5 mm in diameter

as shown in Figure 3b. The smaller tip of the connector was inserted into the sample introduction port at the centre of the graphite tube (Figure 3c). The fused silica tubing protruded by approximately 3 mm from the tantalum tip such that it finally ended 2 mm inside the graphite tube. Precautions needed to be taken to position the tantalum tip so that it did not touch the surface of the graphite furnace, otherwise the security of the HGA-500 power unit shuts down the instrument.

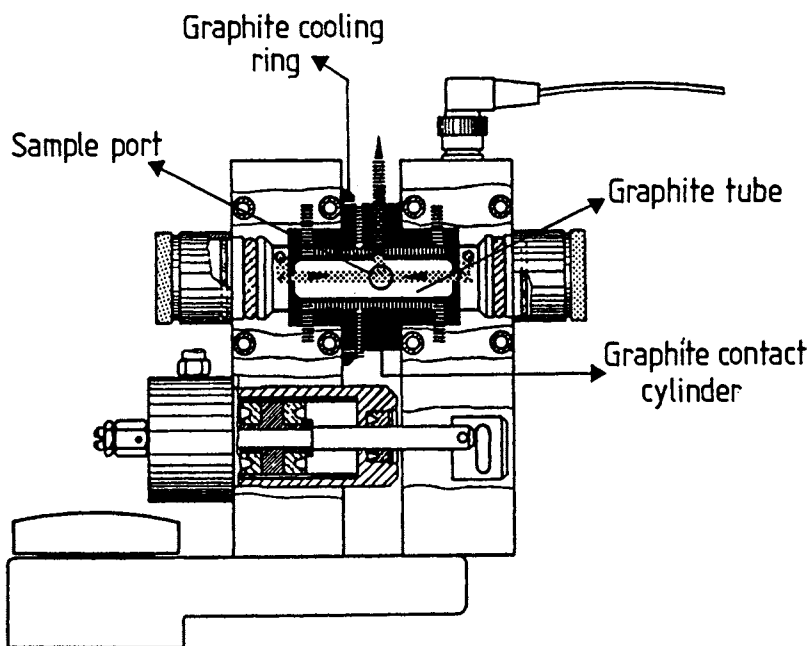


Figure 3a: The modified HGA-500 graphite atomizer

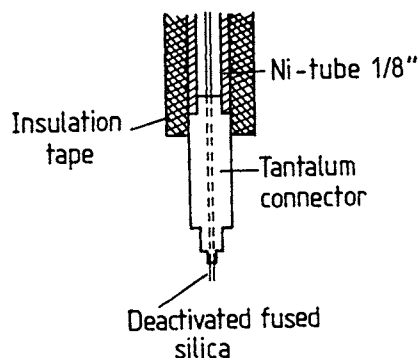


Figure 3b: Details of the Tantalum connector

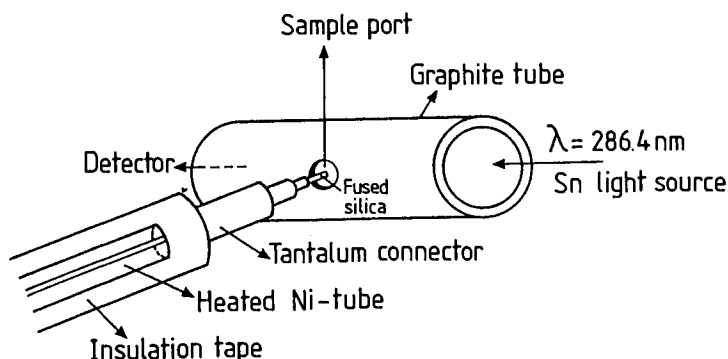


Figure 3c: Tantalum connector positioned in the graphite furnace

15.1.3 Detectors

15.1.3.1 Atomic emission spectrometry

An HP model 5921A atomic emission detector (Hewlett-Packard) was used. The model was equipped with a turbo make-up gas valve and an integrated cooling circuit. The emission source was an atmospheric pressure, microwave-induced plasma sustained in helium. Light emitted by the source is measured with a computerized diode-array spectrometer, which produces element-selective chromatograms.

15.1.3.2 Quartz furnace atomic absorption spectrometry

A PE Model 2380 atomic absorption spectrophotometer (Perkin Elmer, Norwalk, USA) equipped with either a one-slot burner or a PE MHS-20 quartz furnace was used. The radiation source was a tin electrodeless discharge lamp (Perkin Elmer) used at a power of 8 W. Background correction was not used.

The simplest set-up was required for the packed column gas chromatography [45]. A commercially available T-shaped quartz furnace cell (MHS-20) was mounted on the top of a one-slot burner for heating in an air-acetylene flame. Graphite rings (PE 094-413) were attached to the upper tube of the quartz furnace to reduce the noise resulting from the burning of the support gases inside it. The furnace was aligned in the optical beam of the spectrophotometer.

For the megabore column chromatographic detection, the furnace was not heated with the flame but electrothermally using the MHS-20 heating and control unit. Three types of quartz furnace cells: the aforementioned MHS-20 cell, a laboratory modified MHS-20 cell and a FIAS-200 (Perkin-Elmer) cell were used. They differed with respect to the dimensions which are summarized in Table 2.

Table 2: Dimensions of the quartz atomization cells used for AAS in this work.
MHS - Mercury Hydride System
FIAS - Flow Injection Atomic Spectrometry

Cell	Side arm dimensions in mm			Upper tube dimensions in mm		
	o.d.	i.d.	length	o.d.	i.d.	length
MHS-20	8	6	60	14	12	166
Modified MHS-20	6	1.8	50	14	12	166
FIAS-200	8	1.4	40	14	7.3	166

15.1.3.3 Graphite furnace atomic absorption spectrometry

The same AAS instrument but equipped with a PE HGA-500 graphite furnace atomizer pyrolytically coated graphite tubes (Perkin Elmer) was used. The graphite furnace atomizer was modified in a way that the injection hole of the graphite tube was placed horizontally. This was achieved by turning the graphite contact pieces of the furnace by 45 ° as shown in detail in Figure 3.

15.2 Operational conditions

The performance of the overall system critically depends on a variety of parameters which can be classified into three groups:

- (1) gas chromatography related parameters: injection system, design and packing of the liner, injection temperature and vaporization programme, the kind of column used, oven programme, detector port temperature,
- (2) interface related parameters: the material used for the transfer line, dimensions of the transfer line, transfer line temperature, connection to gas chromatographic column and the atomization cell,
- (3) detector related parameters: design and dimensions of the atomization cell, atomization temperature, the choice of support gases, gas flows, furnace programme.

The different approaches examined in this work, are discussed below with particular emphasis given to their advantages or drawbacks. The study on the optimal design and working parameters was performed using the univariate procedure, by repeated injections onto the column of a diluted standard test solution [45,47].

15.2.1 GC Related parameters

15.2.1.1 Injection conditions

The ideal injection system should enable rapid and complete transfer of the analytes onto the gas chromatographic column. Additionally it should allow for the injection of the largest possible amount of the extract without signal distortion to ensure maximum sensitivity.

Since the pentyl (Pe) and butyl (Bu) groups are fairly bulky, the volatility of the organotin species derivatized by pentylation is much lower than that of the species obtained using ethylation (Et), methylation (Me) and, especially, hydride generation. This makes the appropriate injection temperature of paramount importance. Figures 4 a, b and c show the effect of the injector temperature on the response for the injections on a capillary, megabore and packed column, respectively. The curves for a highly volatile compound (Me_3PeSn), one of moderate volatility (Bu_3PeSn) and a high boiling point one (Pe_4Sn) were chosen for comparison. The optimum injection temperatures were similar for all the systems examined and were in the range 160-170°C. Below this temperature a rapid decrease in the response is observed. The sensitivity remains the same for all the compounds irrespective of whether split or splitless injection mode is chosen.

It must be emphasized that the optimum injection port temperatures mentioned above apply only to the calibrant solutions. During routine analysis of environmental samples we noticed that it may be prudent to use more elevated injection temperatures (230 - 260 °C) in order to avoid condensation and progressive accumulation of high boiling point hydrocarbons in the injection liner or in the first 5 cm of the retention gap. The lack of sufficient care in this respect, eventually leads to distorted peak shape, ghost peaks, baseline drift and losses of analytes on the wall of the retention gap tubing resulting consequently in losses of sensitivity.

Sample preparation procedures including a preconcentration step by extraction followed by derivatization have one common drawback. The volume of the organic phase which can be reliably handled is far larger than that tolerated as input by a GC column without affecting the separation of the analytes. In the sample preparation sequence developed in our laboratory, the ionic organotin species are finally preconcentrated by evaporation down to a volume of 250 μl of octane. This is the minimum amount of an organic solvent which can effectively be handled for decomposition of the excess Grignard reagent. Due to the restriction in the column capacity, only a small fraction of the solution available may be injected which results in reduction of the sensitivity of the analytical method. The corresponding dilution factors are 1:10, 1:50 and 1:250 for the packed, megabore and capillary columns, respectively. The large dilution factor for the capillary column represents a considerable drawback.

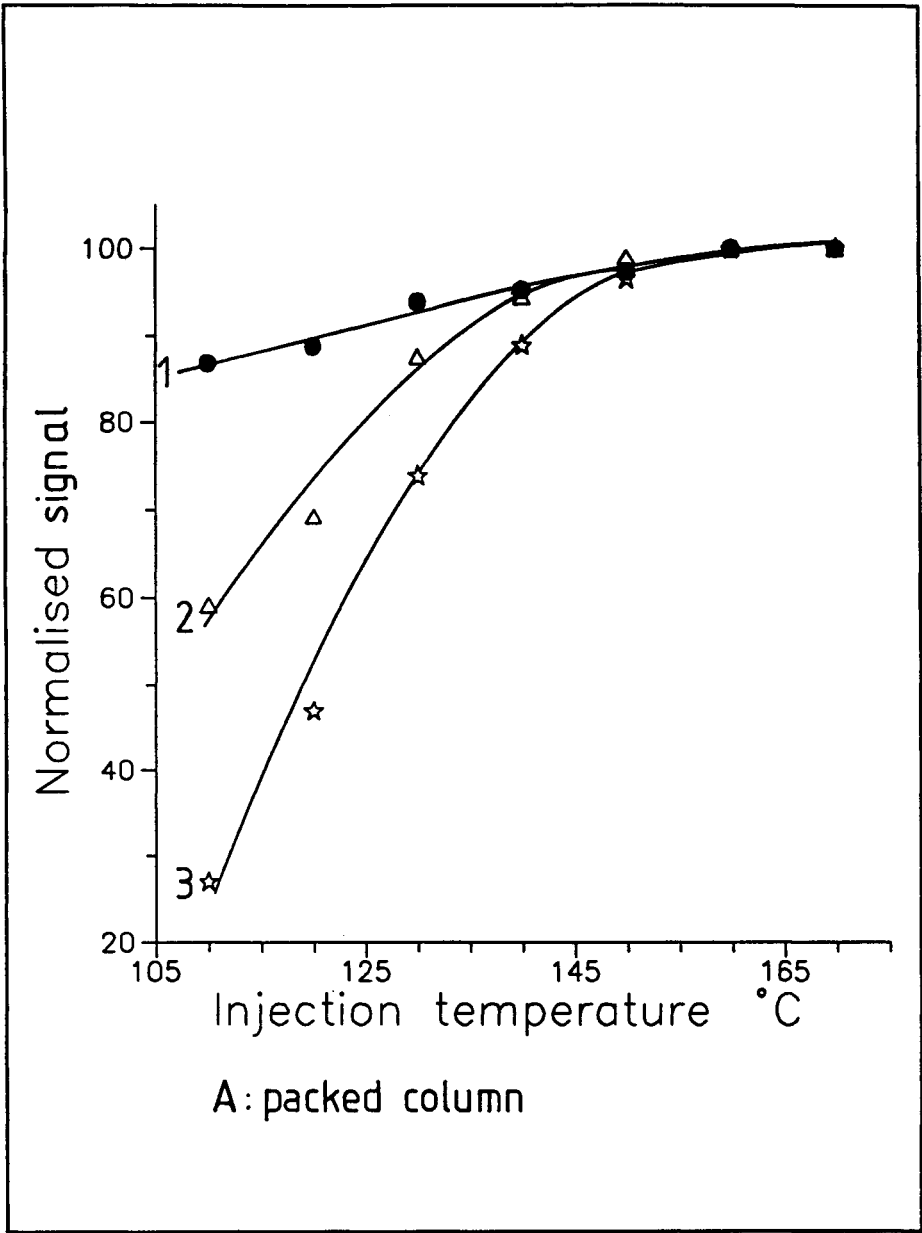


Figure 4a: Effect of injection temperature using different types of injectors:
On column injection (packed column)

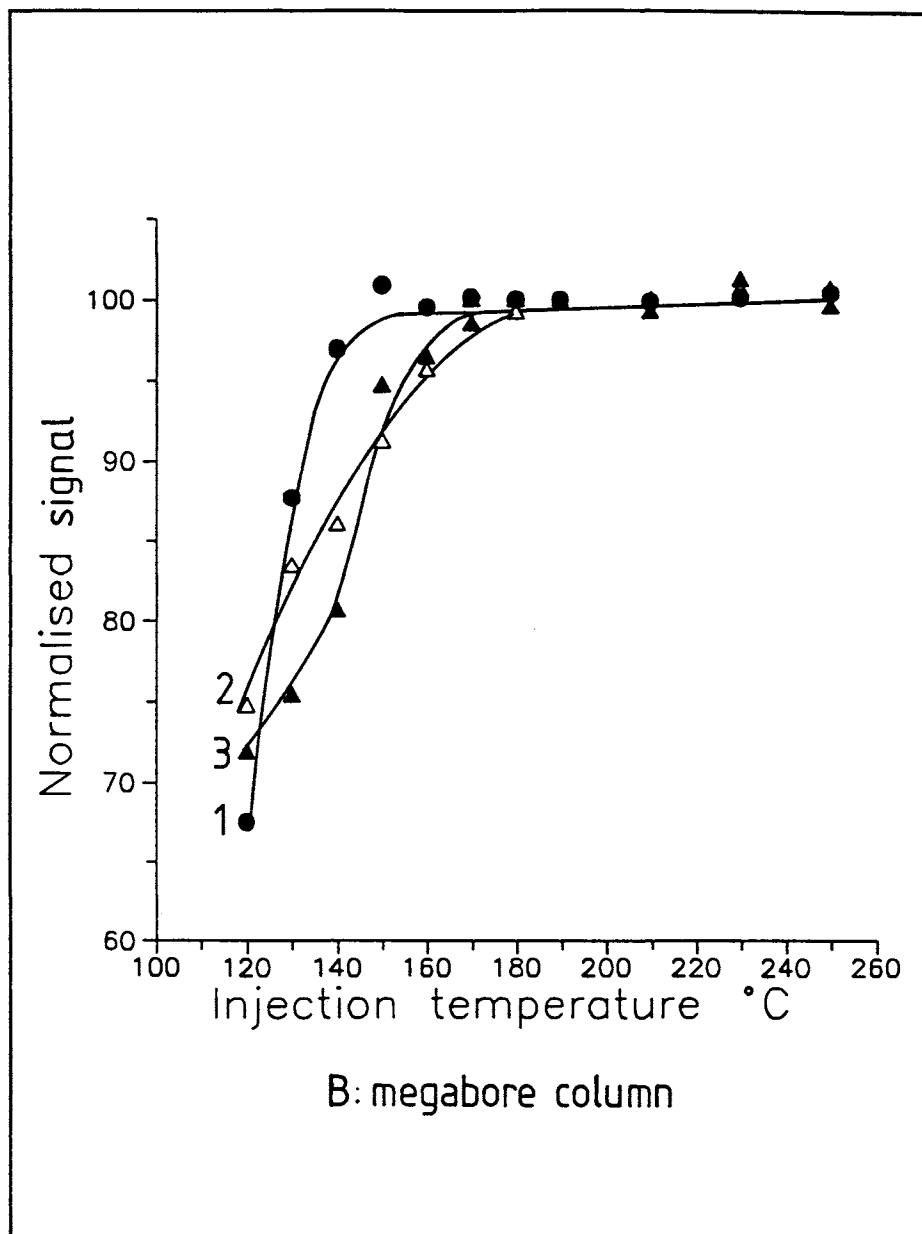


Figure 4b: Effect of injection temperature using different types of injectors:
Wide bore on-column liner (megabore column)

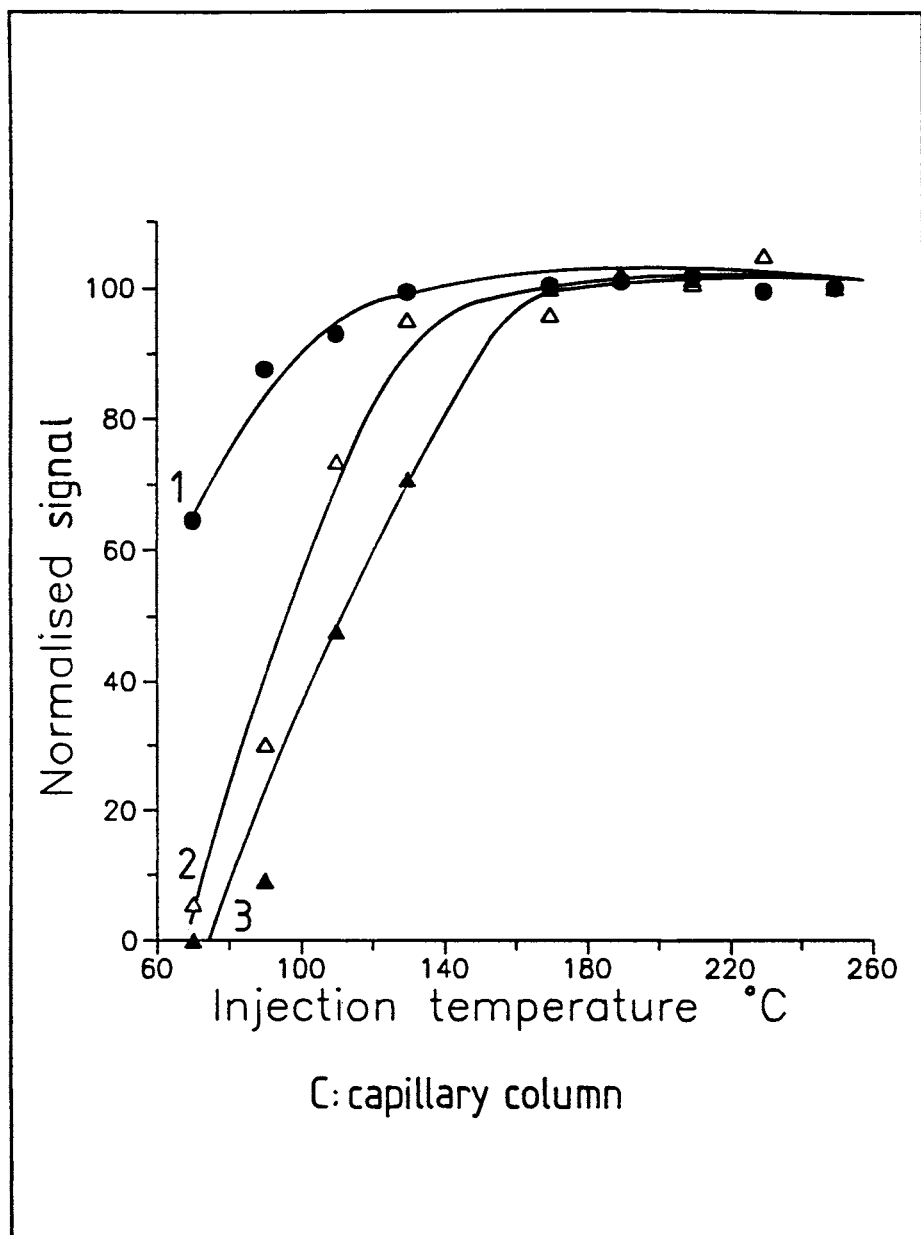


Figure 4c: Effect of injection temperature using different types of injectors:
Hot split/splitless injection (capillary column)
1 - Me_2SnPe ; 2 - Bu_3SnPe ; 3 - Pe_4Sn

Some studies discussing new chromatographic injection techniques to obviate this problem appeared in the literature. They involve large volume injection using on-column retention gap technique [49,50] and in-liner removal of the solvent before the analytes are released onto the column [51]. The first technique is difficult to adapt to MIP-AES detection. It implies that very large volumes of the organic solvent (up to 100 μ l) pass through the column and consequently reach the detector. The GC - AED is equipped with a by-pass valve allowing the solvent vapour to be diverted off the discharge tube but experience shows that it is not sufficient for large volume injections. Indeed, there is a definite possibility of formation of carbon deposits on the wall of the discharge tube with consequently a rapid degradation of the latter. The second approach seems to be more promising and was examined in the present work. The principle of preconcentration is based on the differences in volatility of the solvent and the analytes. Three consecutive processes taking place in the injection liner are involved: sample injection, solvent venting and transfer of the analytes onto the column. A solution of determinands (25 μ l) in a volatile solvent (*e.g.* hexane) is injected onto a cool (0 - 10°C) Tenax packed liner. Then the temperature is slightly raised to increase the solvent vapour pressure and maintained for 1 minute while a stream of helium gas passes through the liner sweeping the solvent off the column. The less volatile analytes are kept in the liner until the volume of the solvent is reduced. Then the purge valve switches the carrier helium gas stream into the column while the temperature of the liner is raised to the effective injection temperature prompting the release of the analytes. The operating conditions for the cool injection programmed temperature vaporization system used are shown in Figure 5.

Figure 6 shows the effect of the packing of the liner on chromatograms obtained for the same mixture of organotin standards. An unpacked liner with swirl holes as well as smooth finished liners but packed with Chromosorb W PH coated with 3 % OV-101 or Tenax were used. The Tenax packed liner produced the best results. The chromatogram for 20 μ l injection shows the same pattern as the one obtained after splitless injection indicating that no losses of analytes occur. When the liner packed with the OV-101 coated Chromosorb was used, the signal from Me_3SnPe was totally lost while the rest of the signals remained unaffected. The use of an unpacked liner leads to losses of methyltin species. Furthermore, the overall sensitivity was lower probably due to the co-sweeping of the analytes with the vented solvent. In all the cases the linearity of the response degraded when more than 30 μ l of the organic phase was injected. Larger volumes may be introduced, however, by repetitive injections, but this results in the drawback of simultaneous preconcentrating all the impurities collected from the sample and the reagents used throughout the sample preparation procedure. This may lead to other pitfalls in the analysis of real samples which will be discussed further in more detail.

Operating conditions for the Cool Injection Programmed Temperature Vaporization GC-AES system

INJECTOR PROGRAMME	
INJECTION TEMP.	15°C
1 st HEAT-UP RATE	2°C.s ⁻¹
SOLVENT REMOVAL TEMP.	25°C
SOLVENT REMOVAL TIME	55 s
2 nd HEAT-UP RATE	12°C.s ⁻¹
ANALYTES RELEASE TEMP.	260°C
ANALYTES RELEASE TIME	60 s
GC PURGE VALVE PROGRAMME	
PURGE VALVE ON	0 - 0.9 min.
PURGE VALVE OFF	0.9 - 2.5 min.
PURGE VALVE ON	2.5 min. - end

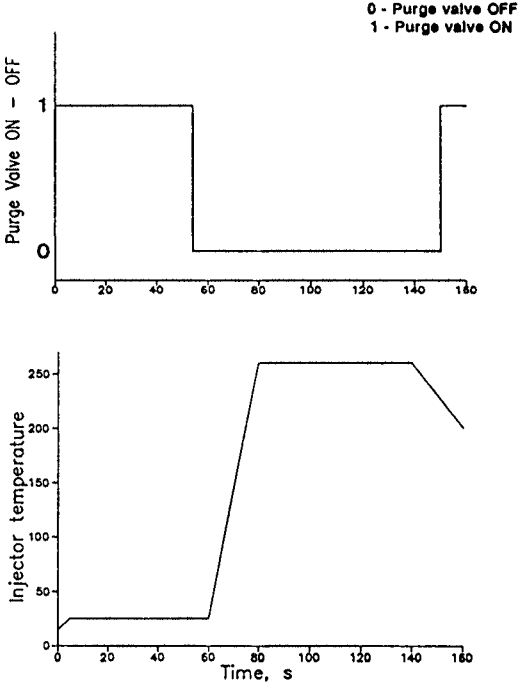
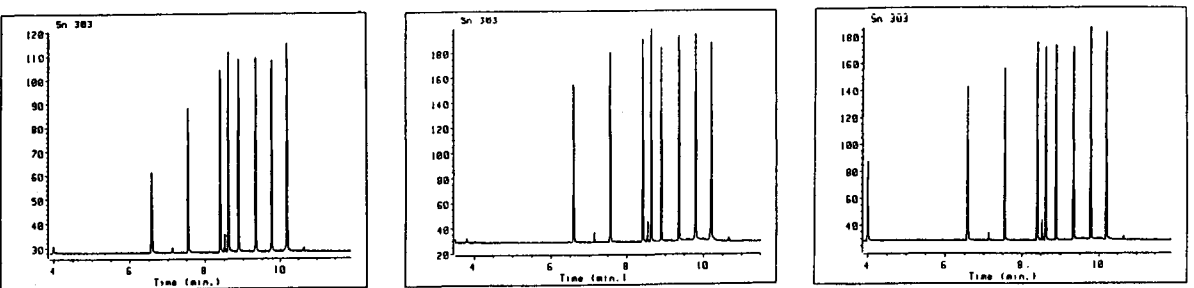


Figure 5:

Operating conditions for the cool injection programmed temperature Vaporization capillary column GC-AES system



Effect of the injection liner on solvent venting during the organotin analysis.

A - smooth finished liner with swirl holes

B - liner packed with OV - 101 on Chromosorb

C - liner packed with Tenax

1 - Me_3SnPe ; 2 - Me_2SnPe_2 ; 3 - Pr_3SnPe ; 4 - SnBu_4 ; 5 - MeSnPe_3 ; 6 - Bu_3SnPe ; 7 - Bu_2SnPe_2 ; 8 - BuSnPe_3 ; 9 - SnPe_4

Figure 6:

Effect of the injection liner on solvent venting during the organotin analysis.

A - smooth finished liner with swirl holes

B - liner packed with OV-101 on Chromosorb

C - liner packed with Tenax

1. Me_3SnPe ; 2. Me_2SnPe_2 ; 3. Pr_3SnPe ; 4. SnBu_4 ; 5. MeSnPe_3 ; Bu_3SnPe ;
7. Bu_2SnPe_2 ; 8. BuSnPe_3 ; 9. SnPe_4

15.2.1.2 Gas chromatographic separation

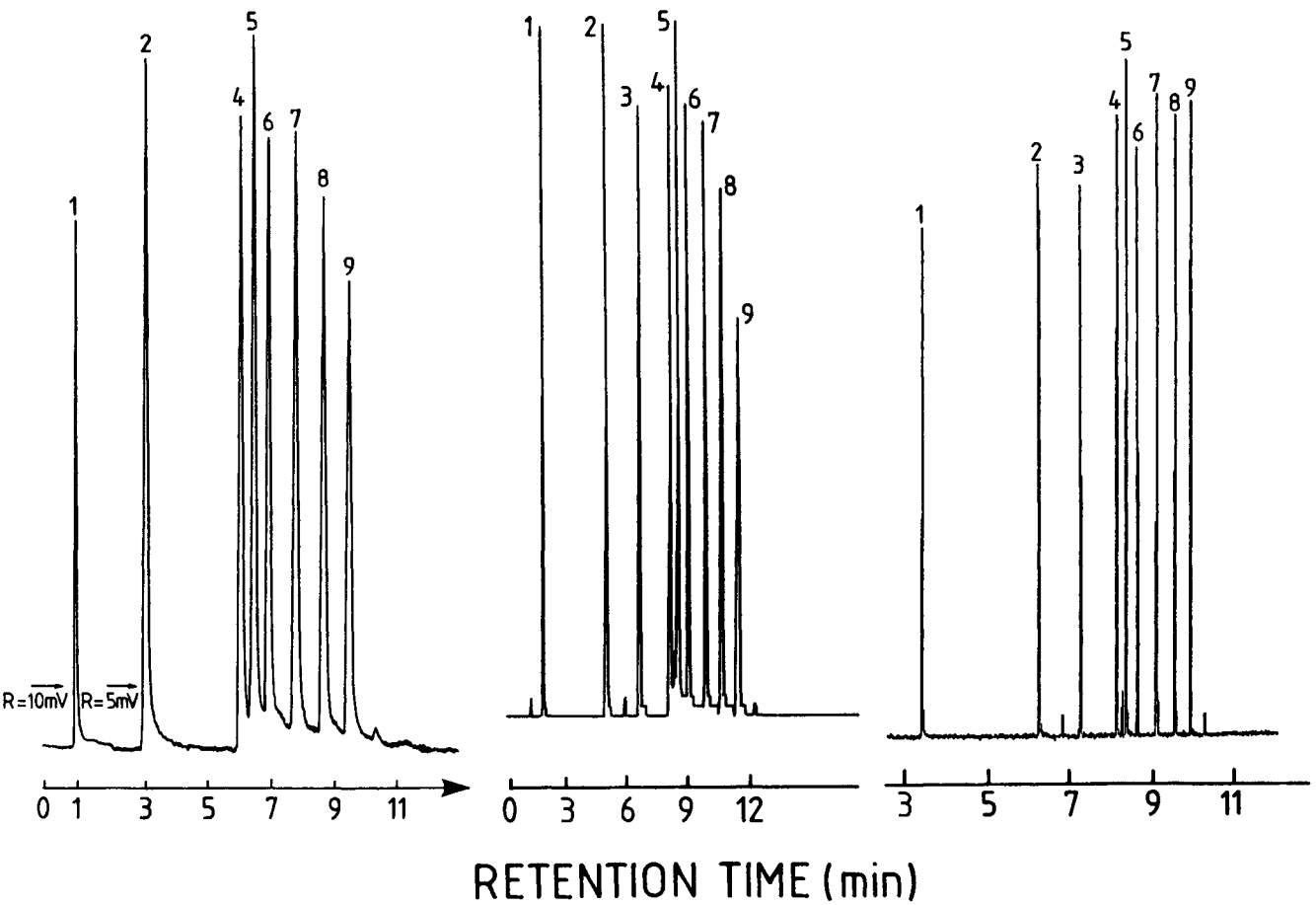
The choice of column not only affects the separation of the analytes but also the peak shape (symmetry and the height to area ratio) thus influencing the sensitivity when the peak height mode is used. Another factor to be considered is the maximum amount of the extract which can be introduced onto the column while still providing clean separation and no peak distortion. Furthermore, each type of column requires a specific detector adapter, design and re-optimization of the operational variables (gas flows and temperatures).

Usually non-polar phases are recommended in the literature for organotin speciation [2]. Light loadings are generally preferred for well defined peaks but insufficient amount of the stationary phase may cause increased tailing. In this work silicone-based stationary phases were used (Table 1).

Figure 7 shows the chromatograms obtained for a mixture of organotin species using packed, megabore and capillary columns. Generally, an adequate resolution may be obtained for most of the analytes on any column, except for problems arising from peak tailing of SnBu_4 , MeSnPe_3 and Bu_3SnPe on a packed column. The peak shape and resolution are significantly better with the capillary column enabling much more sensitive detection of closely eluting compounds almost irrespective of their concentration ratio. Figure 7C shows that the use of a capillary column makes separation possible even for a very small peak of dipropyldipentyltin eluting between the SnBu_4 and MeSnPe_3 peaks. In the chromatogram obtained with a megabore column the dipropyltin peak can also be seen but it is more difficult to quantify. An example comparing the separation of some particular pairs of pentylated phenyl- and cyclohexyltin compounds on different columns is illustrated in Figure 8. In the megabore case, the separation problems are amplified by the condensation effect in other than the GC-AES interface. Hence, capillary columns should be used when these compounds need to be determined.

The effect of the oven heating rate influences peak shape and thus sensitivity. For the packed and megabore columns a value of $10\text{ }^\circ\text{C min}^{-1}$ was chosen as higher heating rates affected the resolution. Proportional increase in the response (in the peak height mode) was observed for a capillary column. A heating rate of $20\text{--}25\text{ }^\circ\text{C min}^{-1}$ was optimum for capillary GC-AED.

The final oven temperature is usually not critical and depends on whether standards or real samples are injected. In the latter case, the final temperature was a minimum of $20\text{ }^\circ\text{C}$ higher than the injection temperature and was maintained during a few minutes to avoid build-up of organic matter in the column. When organotin species with larger substituents, especially the triphenyl and/or tricyclohexyltin, need to be determined the column and the interface must be kept at $300\text{ }^\circ\text{C}$ for 10 minutes to elute these compounds. Such extreme conditions considerably reduce the column lifetime.

**Figure 7:**

Chromatograms obtained for a mixture of organotin species.

A - packed column GC-AAS

B - megabore column GC-AAS

C - capillary column GC-AES

Peaks 1 to 9 as in Figure 6

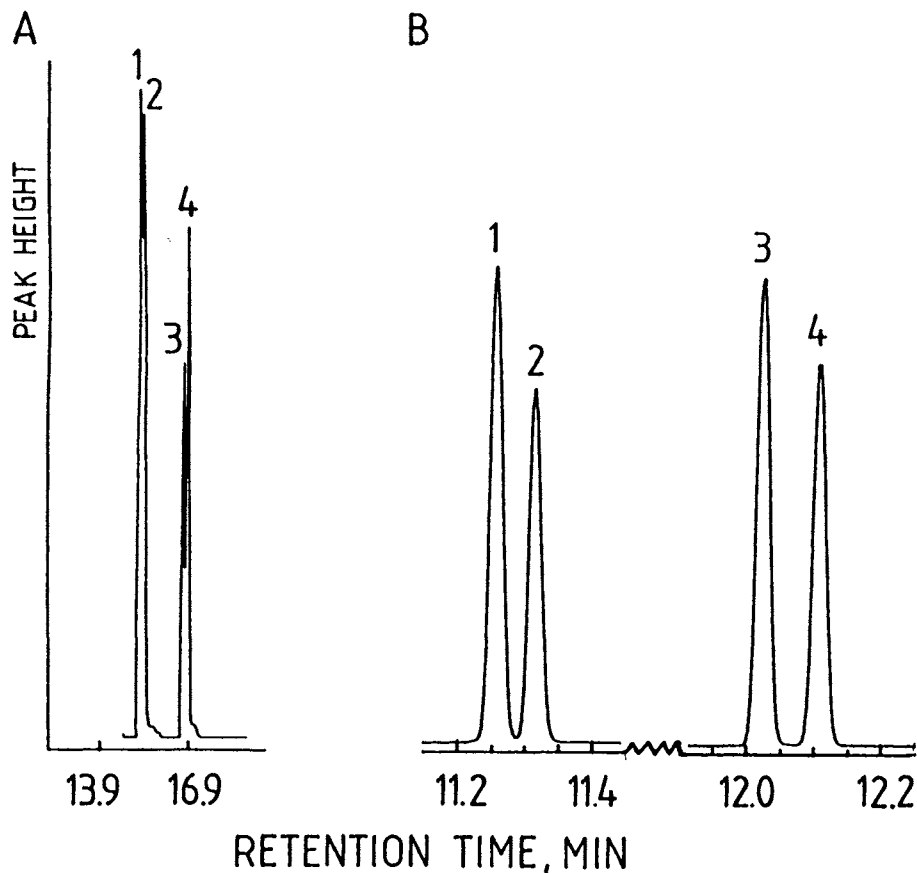


Figure 8: Examples of separation of some organotin compounds on:
A - megabore
B - capillary column
1. Ph_2SnPe_2 ; 2. Cy_2SnPe_2 ; 3. Ph_3SnPe ; 4. Cy_3SnPe

Co-elution of the analytes with the solvent front is an important problem especially in GC-AED. The bulk of the solvent must be prevented from entering the discharge tube otherwise the plasma is extinguished. This is achieved by venting the solvent off the plasma by using a special by-pass valve. A clean separation of the solvent peak from the first analyte peak is also important since the tail of the solvent peak may distort the baseline preventing reliable analysis, especially at levels close to the detection limits. This is why the use of atomic emission detection for megabore or packed column chromatography is difficult unless extremely small volumes of the extract are injected. As the pentylated compounds are much less volatile than species obtained by other derivatization techniques, the use of octane as a solvent results in a solvent peak well separated from most of the analytes, the only exception being

trimethyltin. The signal from this compound may only be seen when a lower boiling solvent, *e.g.* hexane is used. The quartz furnace AAS is more tolerant to solvent vapour but a reasonable difference between the retention times of the solvent peak and the first analyte peak (Me_3SnPe) must be ensured. Here, octane does not interfere with the determination of Me_3PeSn .

15.2.2 Interface design

A properly designed and optimized interface is the most critical part in the coupling of a gas chromatograph to an atomic spectrometer. Ideally, the effluent from the chromatographic column should be introduced directly into the detector atomization cell without any intervening tubing or connectors. In this way sample loss, peak broadening and/or tailing are minimized since the analytes are detected immediately after leaving the column. In practice, however, this approach is not always possible and the column is often connected to the detector by means of a short piece of tubing. This transfer line is heated to avoid any condensation of the organotin species while the temperature is monitored by means of thermocouple. The length of the interface tubing is not critical. It was observed during this work that the proper design of the coupling, careful inspection of dead volumes in the connections used, the right choice and the efficient control of the carrier and support gases, as well as the interface temperature are the most important factors to achieve the optimum system performance (the maximum sensitivity and minimum peak tailing and broadening). This is particularly important in the analysis of organotin compounds derivatized by pentylation. Due to relatively small differences in retention times of the analytes, the determination of all the compounds present is critically dependent upon their separation.

15.2.2.1 Packed column - quartz furnace interface

A system developed in our laboratory and satisfactory applied to the determination of organolead compounds [48] was adapted. After some modification it was used in the beginning of this study [45,47]. A 1-metre piece of nickel tubing of a diameter similar to that of the packed column served as the transfer line. It could easily be heated by applying a transformer-controlled variable voltage between the tube ends. The design included a torroidal transformer which induced a low voltage on the interface. The primary side of this transformer was connected to a variac to facilitate variation and fine tuning of the voltage and thus of the interface temperature. Heating tape could serve the same purpose but it was found to be less convenient in daily practice. The connection of the transfer tubing towards the side arm of the quartz furnace was accomplished in a relatively straightforward manner by means of a Teflon connection part similar to that used in a commercial flow injection analysis (FIAS-200) system. This interface was used in measurements with the quartz furnace heated on top of the one-slot burner.

Peak shape distortions were persistently observed for higher boiling pentylated organotin compounds. They were identified to be due either to decomposition of the analytes before they reached the atomization zone and/or to condensation of high boiling compounds on cold spots on the way to the atomizer. The burner flame produces a large amount of radiant heat from which the interface should be shielded to prevent premature decomposition or atomization of the analytes. The probability that decomposition occurs too early is larger when the column effluent is mixed with

the support gases before entering the atomization zone. Ideally, the analytes should be kept at chromatographic temperatures until they reach the atomization region. This means that the temperature gradient between the end of the nickel tubing and the quartz tube should be as high as possible. Efforts to minimize regions with a temperature higher than the chromatographic temperatures (up to 300 °C) but cooler than atomization temperatures (>900 °C) were made by gas cooling and by shielding the oven. Condensation problems then occurred probably due to the low volatility of the pentylated butyltin compounds.

Adding the furnace support gases via the FID port to the transfer line enhanced the system performance by increasing the laminar velocity of the flow inside the transfer line. However, the condensation problems were mostly associated with the Teflon coupling which warms up by the radiation of the flame. Efforts to heat and to insulate the Teflon connector failed as it melted. In the next step the Teflon union was replaced by a metal reducing union (Figure 2B). The diameter of the side arm of the quartz furnace needed to be adjusted to fit into the 1/4" side of the reducing union. Difficulties with heating the union then occurred. Heating tape was used but proved very inconvenient due to the problems of winding and unwinding the tape whenever there was a need to replace individual parts.

Flame heating of the quartz cell was finally replaced by electrothermal heating which generates less radiant energy and a different approach to the introduction of the support gases and interface was made.

15.2.2.2 Megabore column - quartz furnace interface

The use of 1/8" nickel tubing, as described above, for coupling a megabore column to the quartz furnace would lead to dead volumes, because of the significantly lower diameter of the megabore compared to the packed column. Therefore the use of a section of wide-bore (0.53 mm) deactivated fused silica tubing was examined as the transfer line between the GC column and the quartz furnace. It was pulled through the same 1/8" nickel tubing, as in the case of the packed column - quartz furnace interface. It was connected as described above to the flame ionization detector port which served as the inlet of the support gases for the packed GC-QFAAS equipment. Dead volumes in the connection were easily avoided by the use of a push-and-fit capillary connector. This interface eliminated most problems related to peak tailing and broadening.

Originally, the wide-bore silica tubing was led directly into the quartz furnace of the AAS. This configuration yielded excellent resolution and a far better sensitivity compared to that of the packed column for the most volatile species ($\text{Me}_n\text{SnPe}_{4-n}$, $n = 1-3$). However, tailing, distorted peak shape and the subsequent loss of sensitivity still occurred for higher boiling compounds ($\text{Bu}_n\text{SnPe}_{4-n}$ and SnPe_4). These phenomena were deemed to be caused by the partial condensation of these species on the cold surfaces induced by the addition of the support gases or on cold spots created just in front of the quartz furnace. Therefore modification of the interface design was necessary. A heated block was designed and is shown in detail in Figure 2A. The furnace support gases were added directly into the lower tube of the quartz furnace via a modified reducing union instead of the FID port. In this set-up hydrogen and air were heated before they made contact with the analytes and did not seem to create any cold spot. In addition, the extension of the heated elements almost up to the exterior of the MHS-20 furnace means that the atomization occurs as soon as the analytes enter the

furnace enclosure. As such, the interface enabled effective speciation analysis of the methyl- and butyltin compounds. Smaller condensation problems still remained for the higher boiling pentylated phenyl- and cyclohexyltin species but no effort was undertaken to optimize the interface for these species, too.

15.2.2.3 Megabore column - graphite furnace interface

The use of a graphite furnace atomic absorption spectrometer as the detector for this type of speciation analysis requires a specific approach to the way the column is connected to the graphite furnace as well as the design of the gas supply. The two principal methods of introducing the gas chromatographic column effluent into the graphite furnace consist of:

- (1) forcing the sample gases to follow the flow path that would normally be taken by the purge gas inside the furnace, *i.e.* a symmetrical path from both ends of the graphite tube and escape through the normal sample port inside the tube [11],
- (2) enlarging the sample port of a conventional graphite tube so that the column effluent impinges directly onto the heated graphite furnace [10,12,13].

The first and simplest method was found to be inefficient for the pentylated organotin compounds because of condensation at the cooler parts of the graphite furnace. Difficulties were also encountered with the second approach. As in the case of the GC - quartz furnace interface, the most important aspect of the GC-GFAAS coupling is to heat the transfer line as close as possible to the atomization zone and to avoid a temperature gradient in the final part of the interface. However, specific problems were encountered. The end of the fused silica transfer line needs to be fitted into the graphite tube and therefore the maximum operating temperature allowed is limited by the melting point of the fused silica. It had a tendency to deteriorate or deform at temperatures higher than 1400 °C. Also, since the optimum atomization temperatures are higher in the graphite furnace than in the quartz furnace, additional limitations concerning the thermal conditions of the graphite tube are likely to result in significant drawbacks. We found that when an experiment was conducted at an atomization temperature higher than 1500 °C the fused silica transfer line partly melted inside the graphite tube leading to irreproducible chromatograms. Furthermore, if the end of the fused silica tubing is not properly positioned in the middle of the sample port of the graphite tube, the fused silica can start to deform even at a lower temperature because of the arising tension against the graphite tube wall. This effect leads to a negative baseline drift and drop in sensitivity for the last species eluted. This decline in sensitivity can be explained by an induction of adsorption sites on the deformed fused silica at the sample port or more probably, by the fact that the late eluting species do not reach the graphite tube because of the destruction of the liner. Therefore the fraction of high boiling compounds reaching the wall of the graphite tube is lower.

The use of a connector made of tantalum could circumvent these difficulties. This connector is fitted into the entry port of the graphite tube and allows higher atomization temperatures than were possible with silica. With a tantalum connector, the furnace could be heated to 2500 °C without damage to the transfer line, *i.e.* about

1000 °C higher than in the case of the fused silica. However, direct contact of the analytes with a very hot metal surface induces premature sample decomposition or, if mixtures of compounds are present, disproportionation reactions and even premature atomization in the Ta piece could occur. These phenomena make the positioning of the fused silica inside the tantalum connector of paramount importance. The GC-ETAAS system response was recorded as a function of the position of the fused silica transfer line in the base of the furnace assembly. When the fused silica transfer line ended in the middle of the tantalum connector, no signal was obtained, probably because atomization took place in or at the heated tantalum surface instead of within the optical beam of the spectrophotometer. The highest and most reproducible results were obtained when the fused silica tubing ended approximately 2 mm inside the graphite tube.

15.2.2.4 *The GC-AES interface*

Interfacing a gas chromatograph to an atomic emission spectrometer is more straightforward than to an AAS. This is due to the co-axial position of the column end and the optical axis of the detector. This is not possible in the case of an AAS detector where a light source is needed. The end of the column fits into a discharge tube (the diameter of which is only slightly larger than that of the column) where excitation takes place.

Despite the simple interface, condensation also occurred in GC-AES. Figure 9 shows the effect of the transfer line temperature on the response from a mixture of organotin compounds. A temperature higher than that of the injection is required to minimize condensation. A minimum temperature of 250 °C was sufficient to maintain effective transfer of the gas chromatographic effluent to the detector. In routine analysis it was kept higher (280 °C) to avoid a build up of organic matter within the transfer line.

15.2.3 *Detector design*

15.2.3.1 *Quartz furnace atomic absorption spectrophotometer*

An appropriate design of the atomization cell is one of the crucial factors affecting the sensitivity of analysis [52]. Initially a commercially available MHS-20 quartz furnace was used, but this gave rise to a large dead volume at the position of the connection between the Ni-transfer line and the side arm of the furnace. It was observed that reducing the diameter of the side arm resulted in a large increase in sensitivity. Therefore the MHS-20 atomization cell was modified by reducing the diameter of the side arm in such a way that connection with the reducing union became simple. In the final version the commercial Perkin-Elmer FIAS-200 quartz furnace was used. It was different from the laboratory modified MHS-20 furnace with respect to the internal diameter of the upper and lower tube and the thickness of the tube walls (see Table 2). The increase in sensitivity due to the reduction of the upper tube diameter was also observed but it was very small compared to effect of reducing the diameter of the side arm.

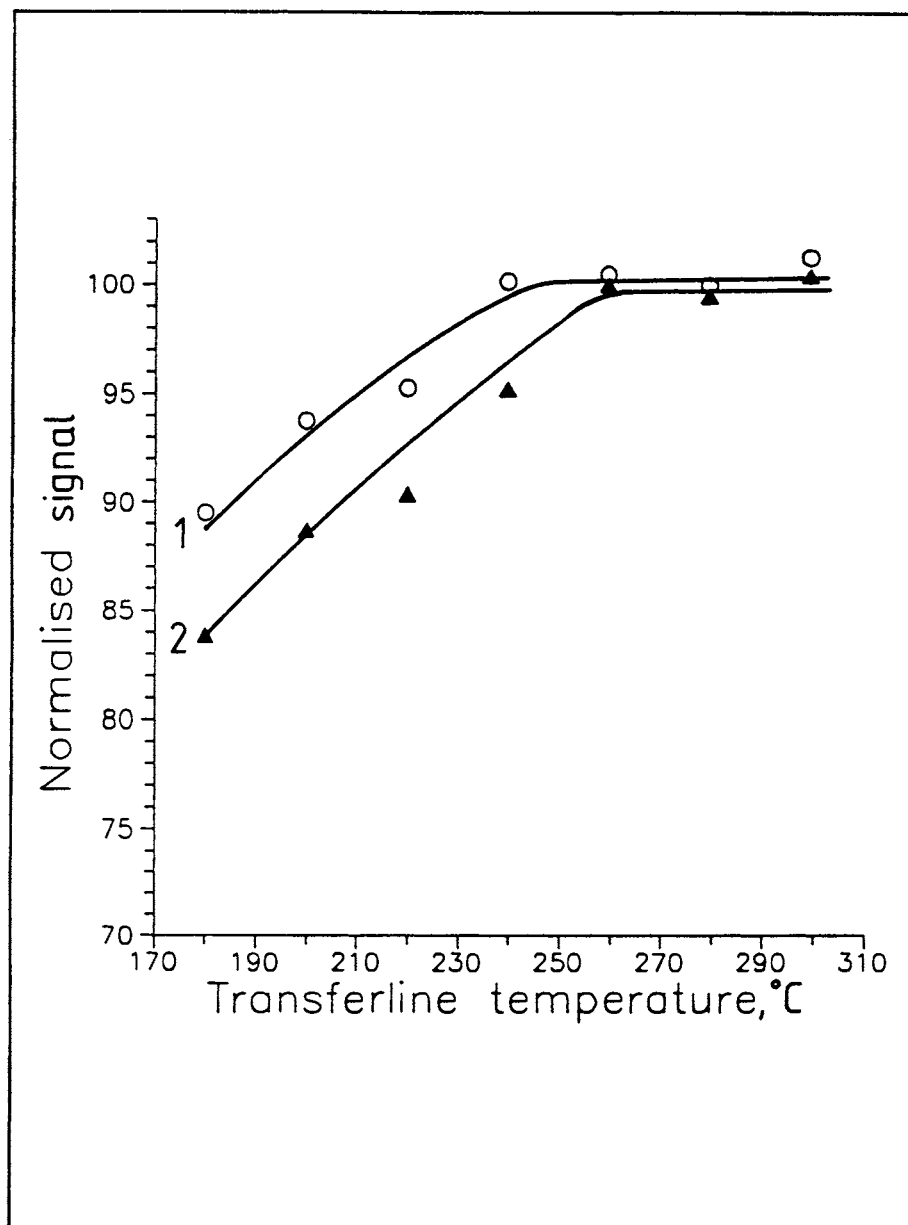


Figure 9: Effect of the transfer line temperature on the response obtained in GC-AES speciation of organotin

It has been documented [8,45,47] that in GC-QFAAS determinations of organotin compounds the peak shape and height are strongly dependent on the flow rates of the furnace hydrogen and air support gases. In the absence of hydrogen virtually no atomization occurs. The beneficial effect of hydrogen on the QF AAS sensitivity for tin is due to two factors. The atomization mechanism is considered to proceed via radicals which need hydrogen to be formed (similarly as reported for lead [52]). Secondly, the combustion of hydrogen in the quartz cell results in a increased furnace temperature. Figure 10A shows the effect of hydrogen on the sensitivity of detection. As only a very small addition of hydrogen results in a dramatic increase in the signal, the role of hydrogen as a catalyst for the formation of tin radicals is more probable than a simple enhancement of the cell temperature. In addition, the shape of the curve is similar to that showing the effect of hydrogen in the MIP-AES determination of tin [14], where the temperature effect is absent. The optimum hydrogen flow rate differs for packed and the megabore columns but the shape of the curve remains the same.

It was also observed that the introduction of air further enhances the sensitivity of organotin determination by GC-QFAAS. Figure 10B shows the effect of the air flow rate on sensitivity. The maximum response is obtained as soon as air is present and remains constant. A flow rate of 10 ml.min⁻¹ is sufficient and increases the sensitivity by a factor of almost 2 (packed column GC-AAS). The optimum values for the make up gases in the GC-QFAAS system equipped with a FIAS furnace are 350 ml.min⁻¹ of hydrogen and 45 ml.min⁻¹ of air.

The flow rate of the carrier gas (argon) also needs to be optimized. Its influence is shown in Figure 10C. Insufficient flow rates result in peak broadening and tailing. Higher flow rates result in deterioration of resolution and reduced sensitivity as the residence time of the atoms in the optical beam decreases. The operating conditions for GC - quartz furnace AAS are summarized in Table 3.

15.2.3.2 Graphite furnace atomic absorption spectrophotometer

The use of a graphite furnace as the detection cell seems to represent an attractive alternative for better sensitivity but effective interfacing is difficult. The introduction of the column effluent into the graphite furnace can be realized through the purge gas inlets [11] or through the sample introduction hole [12,13]. In either case, it is necessary to heat the furnace continuously or very frequently during the chromatographic run. For tin, which requires a high atomization temperature, the furnace tube lifetime is often too short to make the approach viable. Continuously maintaining a high temperature (1500 - 2000 °C) results in premature deterioration of the graphite tube, making the operation of a GC-GFAAS system expensive. To reduce the running costs of the instrument a careful optimization of the graphite furnace program (to match the peak elution times) is necessary. In addition, to ensure high sensitivity and minimum carry-over problems, optimization of the atomization temperature and of the internal gas flow rate was required.

Many problems associated with interfacing of GC and AAS could be overcome when hydride generation sample processing was used [5]. However, when extracts containing less volatile tin species (e.g. phenyl- or cyclohexyltin) need to be analyzed, difficulties associated with condensation and decomposition occur. They result in poor resolution and low sensitivity for these higher boiling compounds.

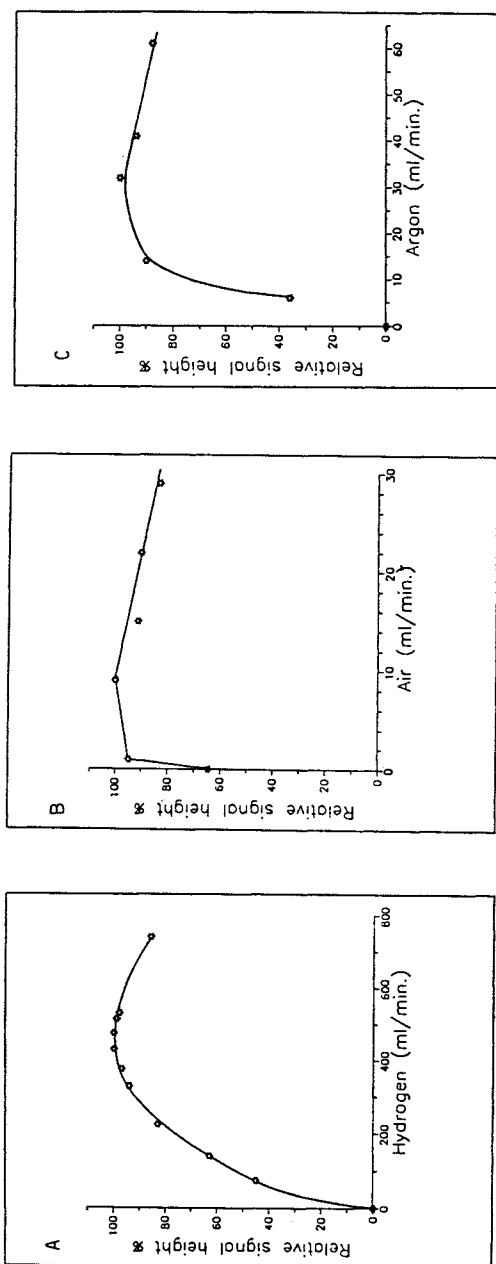


Figure 10: A - Effect of the hydrogen flow rate on the sensitivity of QFAAS detection.
 B - Effect of the air make-up gas on the sensitivity of QFAAS detection
 C - Effect of the argon carrier gas flow rate on the sensitivity of QFAAS detection

Parameters	Packed column GC-QFAAS	Megabore column GC-QFAAS
Injection port	Direct on column	Wide bore on-column liner
Injection port temperature	170 °C	230 °C
Injection Volume	4 - 20 μ l	4 μ l
Carrier gas flow rate	Argon 32 ml/min	Argon 6 ml/min
Oven program	130 °C (10°C/min) 250 °C	100 °C (10°C/min) 260 °C
Transfer line	1/8" Nickel tubing	Deactivated fused silica 530 μ m
Transfer line temperature	250°C	285°C
Atomization cell	MHS-20 Quartz furnace	Fias-200 Quartz furnace
Heating Q cell	Flame	Electrothermal MHS-20 heating unit
Heating block	/	305 °C
Atomization temperature	900 °C	900 °C
Hydrogen makeup gas	470 ml/min.	350 ml/min.
Air makeup gas	9 ml/min.	45 ml/min.
Light source	Sn electrodeless discharge EDL (8W)	
Wavelength	286.4 nm	
Slit	0.7 nm	

Table 3: Operating conditions for GC - quartz furnace AAS.

The graphite furnace program

The operating conditions for GC-graphite furnace AAS are summarized in Table 4. Three steps are required in the operating programme of a GC - GFAAS system. In the first step, the graphite tube is cleaned by purging with argon. During this time the solution of analytes (4 μ l) is injected into the gas chromatographic column. The second step begins when the solvent vapour appears at the entrance of the graphite furnace. The hold time in the first step should be adjusted to match this moment. The eluting solvent peak must be entirely blown out of the graphite tube before the atomization of the analytes begins. This is realized by increasing the internal gas flow rate to 300 ml.min⁻¹ during the second step. The hold time in this step is adjusted to get the best possible resolution between the solvent peak and the first eluting compound (Me₃SnPe). The second step is finished just before the Me₃SnPe peak starts to appear.

By this moment the graphite tube must reach the atomization temperature. The atomization stage (step 3) is the longest step as it is completed only after the last eluting peak is determined. It also makes the graphite furnace program cycle much longer than in a conventional ETAAS analysis. In stage 3 the internal gas flow rate must be reduced; otherwise the analytes are removed too rapidly from the optical beam of the spectrophotometer, to the detriment of detector response.

The lifetime of a pyrolytically coated graphite tube, operated at 1500 °C, ranged between 40 and 50 chromatograms with a duration of 15 minutes. Hence, it is possible to work a whole day with the same graphite tube.

Steps	Temperature , °C	Ramp time, s	Hold time, s	Internal gas, flow ml/min	Comments
1	200	1	10	Ar, 50	Injection
2	200	1	90	Ar/H ₂ , 300	Solvent vent
3	1500	1	730	Ar/H ₂ , 10	Atomization

Table 4: Graphite furnace programme in megabore column - GC - GFAAS measurement. Gas chromatographic conditions were identical with those in Table 3.

Atomization temperature

Chromatograms of a mixed calibrant solution, containing the nine pentylated organotin compounds, were recorded in function of the graphite furnace temperature which was changed from 1350 till 1600 °C. It was experimentally not possible to study atomization at higher temperatures because of the fused silica transfer line. The temperatures indicated were obtained from the HGA-500 power supply settings. The dependence of the peak height on the atomization temperature is very similar for all of the alkyltin compounds, and shows one maximum at 1550 °C. However, after three successive measurements at this temperature the part of the fused silica transfer line inside the graphite tube was irreversibly damaged. Therefore, a slightly lower temperature was chosen. The analyses were performed at an atomization temperature of 1500 °C, which could be maintained for a reasonably long time without damage to the graphite furnace and fused silica transfer line.

Effect of auxiliary gases

As for the quartz furnace the choice and flow rate of the internal make up gas were found to have a strong effect on the absorption signal obtained within a graphite furnace AAS detector. Parris *et al.* [53] reported that the addition of hydrogen to the graphite furnace allows a lower atomization temperature. With a 10 % addition of H₂ to the argon carrier gas a detection limit of 12 ng Sn for tetramethyltin using a bare graphite furnace continuously operated at 1800 °C was obtained.

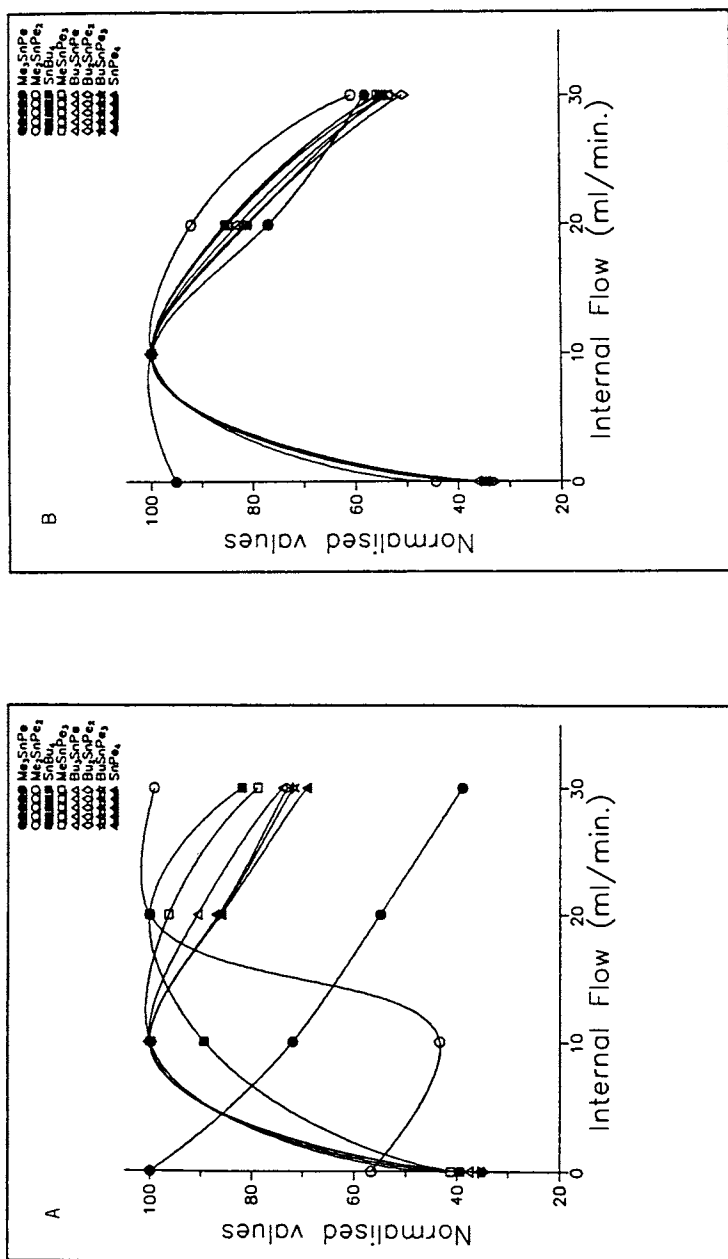


Figure 11: Effect of the internal H_2/Ar flow rate in the step 3 of the graphite furnace program on the absorption intensity of different pentylated organotin compounds.

A - 300 ml.min⁻¹ of argon added in the step 2,

B - 300 ml.min⁻¹ of 10 % H_2 + Ar added in the step 2

The efficiency of atomization (measured as the response observed for a given analyte) on pyrolytically coated graphite tubes was studied for higher boiling organotin compounds. Argon and an argon mixture with 10 % hydrogen were examined as internal flow gases in the step 2 of the furnace program. The flow rate of hydrogen or of the hydrogen doped (10 %) argon was changed from 0 to 40 ml.min⁻¹ at 10 ml.min⁻¹ intervals during the atomization step. Internal flow settings lower than 10 ml.min⁻¹ in the step 3 of the furnace programme cycle were not possible using the HGA-500 power supply. The results are shown in Figure 11.

The shape of the response curve varies depending on whether an inert (Ar) or reactive (10% H₂ in Ar) internal flow gas is used in the step 2. Figure 11A shows that when only argon is used in the step 2 the three first eluting species (Me₃SnPe, Me₂SnPe₂ and SnBu₄) show a completely different behaviour during the atomization than the rest of the pentylated alkyltin species. Me₃SnPe reaches the highest intensity when no gas is added during the atomization step. With the increasing internal flow in the step 3, the response decreases. Me₂SnPe₂ shows a minimum at a flow rate of the H₂-Ar mixture of 10 ml.min⁻¹ but then increases rapidly to reach a maximum at an internal flow rate of 20 ml.min⁻¹. For SnBu₄ an optimum is also achieved at an internal flow of 20 ml.min⁻¹ but no minimum at 10 ml.min⁻¹ is observed. For all other compounds an optimum at 10 ml.min⁻¹ is observed; higher internal gas flow rates in step 3 give lower sensitivity due to the decreased atom concentration in the optical path.

Figure 11B shows that when Ar doped with 10 % of H₂ is used as the internal gas in the step 2, the response from all the pentylated organotin species is maximum at a flow rate of 10 ml.min⁻¹ of the H₂/Ar mixture in step 3. With no internal gas flow, the intensity of Me₃SnPe is 95 % of this maximum while for the other organotin species it is only 30 and 40 %.

Figures 11A and B prove that the composition of the internal gas in step 2 considerably influences the behaviour of organotin species during atomization. From the point of view of application it is important that all the species produce the maximum response for the same experimental settings. This happens only when the argon gas used in the step 2 is doped with 10 % of hydrogen. A continuous flow of the H₂/Ar mixture in the graphite tube could be most easily obtained if this mixture could be used as the carrier gas in the GC. This possibility could not be investigated further since the gas chromatograph used in this work was not equipped with the safety valves necessary to handle hydrogen.

15.2.3.3 Atomic Emission Spectrometer

The commercially available system is described in detail elsewhere [54-56]. Its optimization for the organotin speciation was comprehensively discussed earlier [14,37,47]. The operating conditions are summarized in Table 5.

GC Parameters	
injection port	split/splitless
injection port temp.	170 °C
injection vol.	1 µL
split ratio	1:20
column head pressure	130 kPa of helium
oven program	
initial temp.	60-80 °C
ramp rate	15-20 °C
final temp.	280 °C
Interface parameters	
transfer line	HP - 1 column
transfer line temp.	250 °C
AED parameters	
wavelength	303.419 nm
helium makeup flow	240 ml/min.*
scavenger gases	
H ₂ pressure	50 psi
O ₂ pressure	20 psi
spectrometer purge flow	2 L/min. nitrogen
solvent vent off time	1.5 min.
column-detector coupling	column-to-cavity
cavity temp.	280 °C

Table 5: Optimal GC - AES parameters.

^a Measured at the cavity vent.

15.3 Analytical characteristics

15.3.1 Instrumental detection limits

The instrumental detection limits for GC based techniques are compared in Table 6.

15.3.1.1 Atomic Emission Spectrometry

Microwave induced plasma atomic emission spectrometry is the most sensitive technique for the organotin analysis. The instrumental detection limits reported so far, for the only commercially available configuration, vary considerably between 0.05 pg [14], 0.35 pg [16], 0.5 pg [17] and 6 pg [15] (as Sn). In the latter work apparently underoptimized operating parameters were used. In the former two studies the same detector settings were employed and the same standards injected. The discrepancy in the values reported may result from differences between the systems with respect to individual components (injection system, plasma cooling system) or in differences in definition of the detection limit. For our instrument a down-drift in sensitivity (from 0.05 to 0.20 pg as Sn) was observed during two years of operation [14,22,37,43,47,62]. This decrease could be partly due to the exchange of the waveguide and the pump system. When the baseline is integrated, a standard deviation of 0.08 units is measured. The absolute detection limit calculated as 3 times standard deviation of the baseline varies then between (0.10 - 0.15 pg as Sn). The sensitivity varies on a daily basis; changes are probably related to the condition of the surface of the discharge tube wall. Apart from this effect it was observed that a few injections are necessary to reach a good run-to-run reproducibility, indicating a possible formation of active sites in the system. A better sensitivity (30 - 50 %) was obtained by using the programmed temperature cooled injection system (PTV-CIS) instead of the split/splitless injector. As no signal discrimination was observed in GC - AES measurements, the detection limits can be expressed with one value irrespective of the compound. The sensitivity is considerably higher than that of a GC - AAS system with a gain in sensitivity of about two orders of magnitude.

15.3.1.2 Atomic Absorption Spectrometry

The analytical characteristics for the different techniques involving AAS detection are summarized in Table 7. The absolute detection limits are calculated as three times the standard deviation of the baseline noise. The response is dependent on the molecular mass of the species and the sensitivity decreases towards the higher boiling compounds.

It can be seen that the use of a megabore column and the optimization of the interface brought a significant progress in the detection power of the system for organotin speciation analysis. The instrumental detection limits increased by about one order of magnitude compared to the packed column GC - AAS system. The GC - GFAAS setup is twice less sensitive than the GC - QFAAS equipment. No success was achieved in eliminating the decrease in the response function of the molecular mass of GC - AAS measurements. The sensitivity obtained are consistently about a factor of two lower for the late eluting compounds than for the first ones irrespective of the interface used. It is not clear whether this is only due to condensation problems in the transfer line and the interface, or also to non quantitative injection occurring at the same time.

Detector	Instrumental setup	Definition	Detection limit pg as Sn	Ref.
FPD	Column: Megabore column [57], capillary column [21,24,26]	3σ	1.2*	24
		2σ	1.1*	26
		2σ	30	57
			0.016*	58
			0.2	21
QFAAS	Column: Packed column [4,25,59], megabore column [8,14,37,47], capillary column [60] Transfer line: Metal tube [23,45,59], fused silica tube [8,25,37,60] Interface: Modified reducing union with support gas inlets [8,25]. Made Teflon union [45]	Mean $N_{pp} + 3\sigma$	7.3 - 17.2	8
			4.8 - 13.8	25
		3σ	17.5 - 37.2	14
		3σ	160 - 400	45
			80 - 370	59
CT - QFAAS	Column: U-shaped trap, 3% SP-2100 on Chromosorb Transfer line: Teflon tubes Interface: Teflon connectors	3σ	30	5
		3σ	11 - 45	
GFAAS	Column: Megabore column [13] Transfer line: Deactivated fused silica tubing in heated metal tube Interface: Tantalum connector			
		3σ	33 - 71	13
MIP - AED	Column: Fused silica capillary column [14-17, 22,37,43,46,47,61] Transfer line: Column in heated metal tube [14-17, 22,37,43,47] Interface: Column to discharge tube in cavity	3σ	0.05	14
		3σ	0.10 - 0.15	37
		3σ	0.15 - 0.2	22
		2σ	6.1	61
		3σ	6	15
			0.35*	16
			0.5	17

Table 6: Comparison of detection limits reported for hyphenated techniques used in the speciation analysis of organotins.

* Recalculated, in original was given per compound.

Mean N_{pp} : Mean peak to peak baseline noise.

σ : Standard deviation

Table 6:	Abbreviations
	FPD: Flame Photometric Detection
	QFAAS: Quartz Furnace Atomic Absorption Spectrometry
	CT-QFAAS: Cryogenic Trapping Quartz Furnace Atomic Absorption Spectrometry
	GFAAS: Graphite Furnace Atomic Absorption Spectrometry
	MIP-AED: Microwave Induced Plasma Atomic Emission Detection

Analyte	Retention time min.	Absolute Detection Limit* (pg Sn)	
		GC-QFAAS	GC-GFAAS
Me ₃ SnPe	1.69	17.5	32.6
Me ₂ SnPe ₂	4.78	28.2	57.2
Pr ₃ SnPe	6.43	35.2	/
SnBu ₄	7.95	35.4	70.1
MeSnPe ₃	8.36	37.2	70.7
Bu ₃ SnPe	8.83	34.0	62.6
Bu ₂ SnPe ₂	9.68	35.3	68.0
BuSnPe ₃	10.47	35.2	66.6
SnPe ₄	11.25	36.7	66.4

Table 7: The absolute detection limits of the GC-QFAAS and GC-GFAAS systems.
 * Amount of Sn per species required to give a signal 3 times the standard deviation of the baseline noise.

15.3.2 Experimental detection limits

For the analysis of real samples, *e.g.* environmental water samples, except from the instrumental detection limit a number of other parameters determine the experimental detection limits. Apart from the sample intake for analysis (up to 3L) and the preconcentration factor (more than 10.000), the volume of the organic phase that can be injected is important. The gain in instrumental detection power for the systems using capillary and megabore columns is partly levelled by the smaller sample volume which can be injected. Large volume injection techniques may be a remedy but upon preconcentration by evaporation, interferences may affect theoretical detection limits. The possible interferences will be discussed in section 6.2. Table 8 shows a comparison of the experimental detection limits for different hyphenated techniques.

Hyphenated technique	Injected volume (μ l)	Instrumental DL* in pg as Sn	Relative DL** in ng/l as Sn
PC GC - QFAAS	20	160 - 400	0.7 - 1.7
MC GC - QFAAS	4	17 - 37	0.35 - 0.8
MC GC - GFAAS	4	33 - 71	0.7 - 1.5
CC GC - AES	1	0.10 - 0.15	0.008 - 0.013***
PTV CC GC - AES	25	0.10 - 0.15	0.0003 - 0.0005***

Table 8: Detection limits obtained in this work using different hyphenated techniques.

* Amount of Sn per species required to give a signal 3 times the standard deviation of the baseline noise.

** Based on a sample volume of 3l.

*** Affected by blank contributions from the Grignard reagent.

PC Packed column

MC Megabore column

CC Capillary column

PTV Programmable Temperature Vaporization

GC - QFAAS Gas chromatography Quartz Furnace AAS

GC - GFAAS Gas chromatography Graphite Furnace AAS

GC - AES Gas chromatography Atomic Emission Spectrometry

15.3.3 Reproducibility

The precision of the measurements was studied by replicate injections of mixed calibrant solutions at different Sn concentration levels under optimized conditions (Tables 2-5) using the peak height mode for quantification. The precision of megabore column GC - QFAAS and GC - GFAAS is comparable. On the basis of ten replicate measurements at a level of 4 - 8 ng Sn injected analytes, a relative standard deviation of 2 - 4 % and 3 - 7 % was obtained. Similar values using the packed column GC - AAS system, are at a level of about 100 - 200 ng. In GC - AES measurements the precision is similar for all the compounds determined and reaches less than 5 % at the pg level. At a 20 - 50 pg level a standard deviation of 1 - 2 % can routinely be obtained.

15.3.4 Dynamic range

The linearity of the response in the developed hyphenated techniques was measured by injecting different concentrations of the mixed standard solution of organotin compounds. The widest dynamic range extending well over three decades was observed in GC - AES measurements. Larger concentrations were not examined as these might result in an overloading of the discharge tube, followed by its rapid deterioration and the appearance of a noisier baseline. Linear ranges up to 15 - 30 ng and 60 ng (as Sn) were obtained for QFAAS and GFAAS, respectively.

15.4 Sample handling

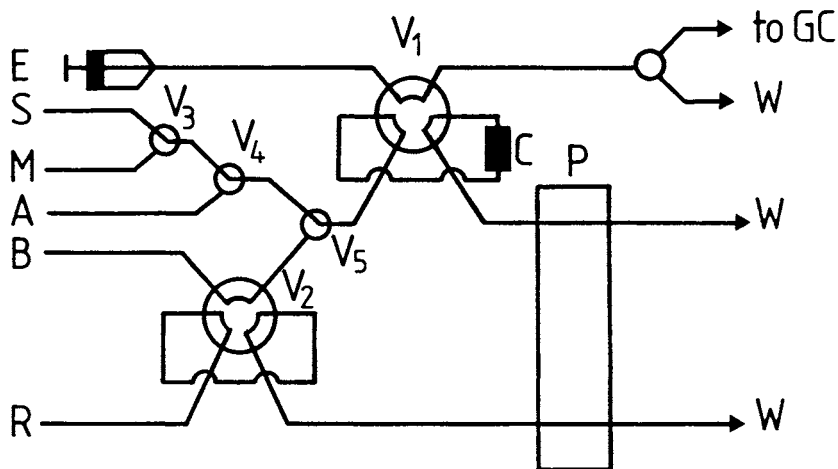
15.4.1 Water samples

Earlier methods based on acidification with hydrochloric (HCl), hydrobromic (HBr) and acetic acid (HAc) of the sample to release alkyltin compounds from the suspended particulate matter and to convert them into the respective halogenides which were then extracted into a variety of organic solvents usually of relatively high polarity. This approach usually succeeds for trisubstituted organotins (TBT, TPhT) and tricyclohexyltin (TCyT) but fails for other species due to their higher polarity. In addition the more polar solvents are poorly compatible with the Grignard reagents used later for derivatization and favour co-extraction of organic interferents. Therefore the currently recommended procedures base on the extraction of low polar organotin complexes with tropolone or diethyldithiocarbamate (DDTC) using a non polar solvent. Tropolone is preferred to DDTC as under acidic conditions ($\text{pH} < 4$) the latter undergoes decomposition, giving rise to extractable interferences [13].

An elegant approach which is rapidly gaining popularity is extraction of the analytes derivatized earlier *in-situ* [19-23]. Sodium tetraethylborate (NaBEt_4) is preferred to NaBH_4 as a derivatization reagent. Hydride generation is more prone to interferences and in case of monosubstituted organotins it leads to very volatile derivatives which can hardly be further preconcentrated by evaporation of the extraction solvent. In addition organostannanes are relatively reactive and tend to decompose when subject to cleanup or harsh instrumental conditions.

Liquid-liquid extraction methods often require large amounts of hazardous solvents and tend to be replaced by the solid phase extraction (SPE) procedures [26-28,35,43,44]. The advantages of the SPE include a higher preconcentration factor and ease of application in the field and in on-line systems while a drawback is that only filtered samples can be analyzed. Recently, Szpunar-Lobinska *et al.* [43] developed a semi-automated flow-injection system for on-line sample preparation and determination of mono-, di- and tributyl and phenyltin compounds in water. The procedure is based on the preconcentration of ionic organotin compounds by sorption on bonded silica with C_{18} functional groups followed by on-column ethylation using NaBEt_4 . The derivatized species are eluted with 250 μl of methanol, separated and detected by GC-MIP-AED. The flow-injection manifold for on-line integrated preconcentration/derivatization of ionic butyl- and phenyl tin species in different operation modes is shown in Figure 12; the experimental conditions were also summarized and figures of merit available in the literature on the application of solid-phase extraction in the speciation analysis of organotin [43]. Similar as Clark *et al.* [59], Dowling [17] injected preconcentrated solid phase extracted alkyltin chlorides in dichloromethane through an injection port liner packed with 100 mg of NaBH_4 . The respective organotin hydrides were formed on-line and detected with GC - AED. They showed that on-line hydride conversion improved chromatographic behavior and analyte recovery. Table 9 summarizes the methods enabling the quantitative recovery of organotin compounds in comprehensive speciation analysis of water.

I. Preconcentration



II. Derivatization

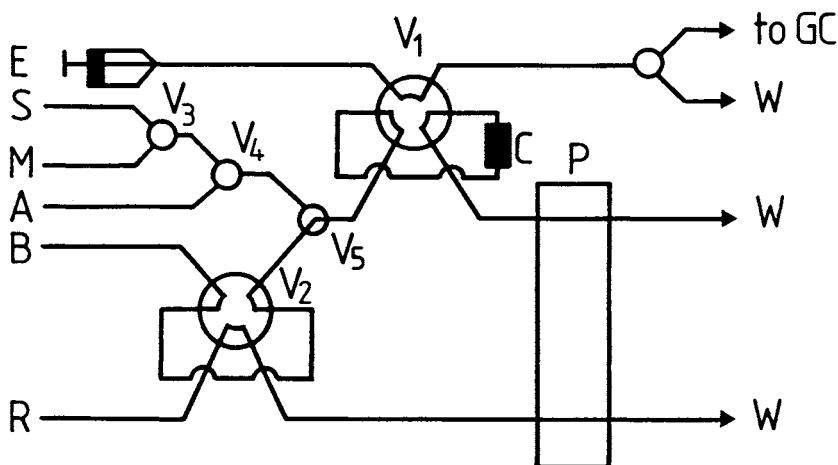
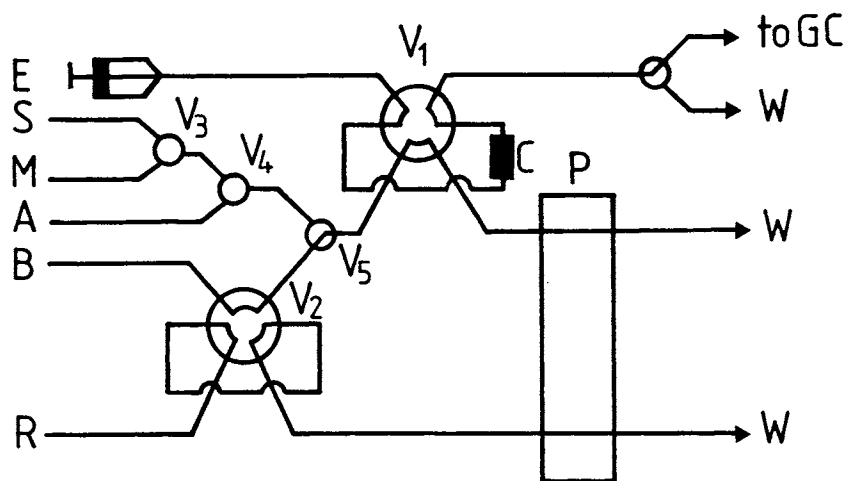


Figure 12: Flow manifold used in on-line preconcentration-derivatization of organotin compounds.

B= Buffer; E= Eluent; S= Sample; R= derivatizing agent; M= methanol; A= air; P= peristaltic pump; V₁ and V₂= six-way valves; V₃ and V₆= three-way valves; C= preconcentration microcolumn; D= to detector; W= to waste

III. Elution



IV. Washing

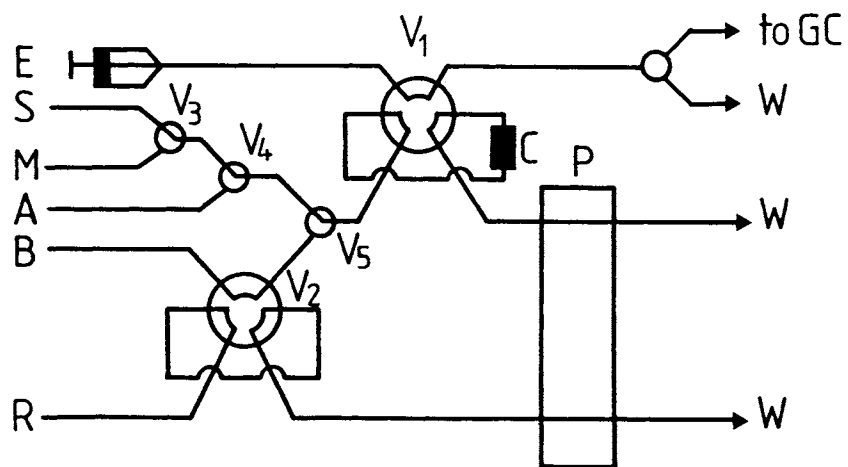


Figure 12 (ctd)

Extraction reagents (Solvent)	Derivatization agent	Experimental detection limit (ng.L ⁻¹ Sn)	Species (% Recovery)	Ref.
Washed C ₁₈ Sep-Paks (dichloromethane)	NaBH ₄ in inj. port liner	MIP-AED (0.5)	TBT, TPrT (90)	17
NaBH ₄ (dichloromethane)	NaBH ₄	FPD (1)	TBT,DBT,MBT (>95)	20
acetic acid/acetate, pH = 5, NaBEt ₃ (hexane)	NaBEt ₃	MIP-AED (0.1)	TBT,DBT,MBT (85 - 97) TPhT,DPhT,MPhT (85 - 97)	22
Tris/acetic acid, pH = 6, NaBEt ₃ (iso-octane)	NaBEt ₃	FPD (0.4)	TBT,DBT,MBT (>95)	21
tropolone (dichloromethane)	PeMgBr	FPD (2)	TBT,DBT, MBT TPhT,DPhT,MPhT	34
HCl, tropolone (hexane)	MeMgCl	FPD (0.5 - 6.5)	TBT (61 - 93), DBT,MBT TPhT (56 - 89), DPhT,MPhT	24
HCl, ascorbic acid, Sep-PAK C ₁₈ cartridge, tropolone (diethyl ether)	EtMgCl	FPD (0.01) FPD (3.5 - 8) FPD (17 - 51)	TBT,DBT,MBT (60 - 95) TPhT,DPhT,MPhT (60 - 95) TCyT,DCyT,MCyT (60 - 95)	26-28
HBr, tropolone (pentane)	PeMgBr	FPD (20 - 50)	TBT,DBT,MBT (>90) TPhT,DPhT,MPhT (>90)	36
HCl, NaCl, tropolone (hexane), 1h	EtMgCl	QFAAS (5)	TBT,DBT,MBT (84 - 95 ± 6) TMT,DMT,MMT	29
HCl, pH = 1, NaCl, tropolone (toluene), 4h	EtMgCl	QFAAS (40)	TBT (90 - 104), DBT (99 - 109), MBT (101 - 109)	30
HCl-THF (1+3), NaCl, tropolone (benzene)	PrMgCl	FPD (3)	TBT,DBT,TPhT,DPhT (92 - 100) MBT,MPhT (70)	32
citric acid/phosphate, pH = 5, NaDDTC (pentane)	PeMgBr	QFAAS (0.36 - 0.78) MIP-AED (0.008 - 0.013)*	TBT,DBT,MBT (101 - 105) TMT,DMT,MMT (101 - 106)	14, 37
C ₁₈ , citrate-ammonia pH = 9, NaBEt ₃ , (methanol)	on-column NaBEt ₃	MIP-AED (0.1 - 0.2)	TBT,DBT,MBT (80 - 100) TPhT,DPhT,MPhT (80 - 100)	43
Carbopack or LC ₁₈ , H ₂ O, tropolone (methanol)	PeMgBr	FPD (2) GFAAS (10)	TBT,DBT,MBT (98 - 100) carbopack TBT,DBT,MBT (94 - 102) LC ₁₈	35

Table 9: Selected methods for organotin speciation analysis in water^a.

a TBT, DBT, MBT: tri-, di and monobutyltin; TPhT, DPhT, MPhT: Tri-, di and monophenyltin; TCyT, DCyT, MCyT: tri, di and monocyclohexyltin; TPrT: Tripropyltin.

* Based on sample volume of 3 l

15.4.2 Sediment samples

As organotin compounds are not involved in mineralogical processes and bind onto the surface of the sediment the complete dissolution of the latter prior to the analysis is not considered necessary. The basic approach to release organotin compounds from the sediment involves acid leaching (HCl, HBr, HAc) in an aqueous or methanolic medium by sonication, stirring, shaking or Soxhlet extraction with an organic solvent. To increase the extraction yield the addition of a complexing agent (tropolone, DDTC) is mandatory.

In recent years, supercritical fluid extraction (SFE) has gained popularity as a sample preparation technique. Compared with conventional solvent extraction, SFE is relatively fast, and its selectivity can be easily controlled. In addition, carbon dioxide, the supercritical fluid most commonly used, is nontoxic, nonflammable, and relatively inexpensive. Liu and coworkers [63] were the first, who tried to extract organotin compounds from spiked topsoil samples with supercritical fluid extraction followed by pentylation and GC-AED detection. Recoveries ranging from 70 to 90 percent were obtained for most organotin compounds when NaDDTC was added to the samples. These results show that SFE-GC-AED is a promising technique for the determination of trace organotin compounds in environmental solid samples. The development of SFE for tin speciation analysis is described in details in Chapter 18 of this book.

Table 10 gives a survey of analytical procedures for speciation analysis of organotins in sediments claimed to give the highest recovery. Apparently no reliable and efficient method for extracting all organotin species simultaneously from sediments has hitherto been developed. While the tri- and disubstituted compounds can be extracted quantitatively, only about 60% or less of the mono substituted compounds are recovered. The reason for this is the uncomplete extraction and/or derivatization because of a relatively high polarity of monobutyltin (MBT) and its adsorption affinity to the components present in the sediment. The recovery is usually higher for butyltins than for methyltin compounds.

15.4.3 Biological materials

Prior to GC separation, the organotin compounds need to be liberated from the biological matrix, followed by a derivatization reaction. Extraction of organotin compounds after acid leaching from the biological matrix followed by Grignard derivatization [25,28,38,64-69], hydride generation [70-74] or NaBEt₄ [75] are the most common approaches. An alternative to acid leaching is the use of tissue solubilizers [76,77] (e.g. TMAH) or enzymes [25,65-67] (e.g. lipase-protease mixture) to decompose the sample matrix. The use of enzymes or TMAH does not impose a problem for altering the chemical structure of the organotin analytes, in contrary to methods based on acids where care must be taken to avoid degradation [78]. Ceulemans *et al.* [75] recently revisited the different approaches for breaking down the biological matrix of a reference fish tissue (NIES Nr 11), followed by a rapid *in-situ* NaBEt₄ derivatization and GC-AED measurement. His method allowed an accurate determination of butyl- and triphenyltin compounds down to the level of 2 ng.g⁻¹ (as Sn). Table 11 summarizes a selection of sample preparation methods used for organotin speciation analysis in biological samples.

Extraction reagents (Solvent)	Derivatization agent	Experimental detection limit (ng.g ⁻¹ Sn)	Species (% Recovery)	Ref.
HCl/MeOH (cyclohexane) reflux 80°C	NaBH ₄	FPD (50)	TBT (79 ± 26) DBT	19
0.1% NaOH/MeOH (hexane)	NaBH ₄	FPD (10)	TBT,DBT,MBT	20
HCl, tropolone (dichloromethane)	NaBEt ₃	QFAAS	TBT,DBT,MBT (94) TPhT,DPhT,MPhT TCyT,DCyT,MCyT	23
MeOH-HCl, tropolone, sonication (toluene/isobutyl acetate)	—	FPD (30)	TBT (94 ± 5), DBT (95 ± 2), MBT (86 ± 4)	57
HCl, tropolone (diethyl ether)	EtMgCl	FPD (0.01) FPD (0.02-0.7)	TBT,DBT,MBT (60 - 95) TPhT,DPhT,MPhT (60 - 95) TCyT	26-28
	MeMgCl	FPD (0.1 - 2)	TBT (61 - 93), DBT,MBT TPhT (56 - 89), DPhT,MPhT	24
HCl, HBr, tropolone (pentane)	PeMgBr	FPD (3)	TBT,DBT,MBT	38
tropolone (dichloromethane)	PeMgBr HeMgBr	FPD (5) FPD (5)	TBT (94 ± 7) DBT (97 ± 16) MBT (40 ± 23)	39,40
H ₂ O, NaCl, KI, sodium benzoate, tropolone (hexane)	EtMgCl	QFAAS (5)	TBT,DBT,MBT (84 - 95 ± 6) TMT,DMT,MMT	29
HCl, NaCl, tropolone (toluene), 4h	EtMgCl	QFAAS (2)	TBT (90 - 114), DBT (89 - 95), MBT (96 - 103)	30
HCl-THF (1+11), tropolone (benzene)	PrMgCl	FPD (0.5)	TBT,DBT,TPhT,DPhT (92 - 100), MBT,MPhT (70)	32
H ₂ O, acetic acid, NaDDTC (hexane)	PeMgBr	QFAAS (0.45) MIP-AED (0.005-0.075)*	TBT (95 - 105) DBT (95 - 107) MBT (30 - 45)	14,37 ,47
SFE, Topsoil CO ₂ CO ₂ + 5 % MeOH	PeMgBr	MIP-AED	TMT,TET,TBT (50 - 70) TMT,TET,TBT (50 - 70)	63
SFE, Topsoil + NaDDC CO ₂ CO ₂ + 5 % MeOH	PeMgBr		TMT,TBT,DMT,DBT (70 - 90) TMT,TBT,DMT,DBT (70 - 90)	

Table 10: Selected methods for organotin speciation analysis in sediments.

* Based on 5 g sample volume

Table 11: Selection of analytical procedures used for organotin speciation in biological samples

Analytes	Sample	Sample preparation	Extraction & derivatization	Separation & Detection	Detection limit (ng/g as Sn)	Reference
Me _n Sn ⁽⁴⁻ⁿ⁾⁺ Bu _n Sn ⁽⁴⁻ⁿ⁾⁺	oyster	MeOH / HCl 7 mol.l ⁻¹ 1 h ultrason 60°C	NaBH ₄	thermal desorption QFAAS	11 - 25	71
Bu _n Sn ⁽⁴⁻ⁿ⁾⁺	oyster	HCl 2 mol.l ⁻¹ 12 h leaching	NaBH ₄	GC - QFAAS	0.5 - 3.5 (absolute)	70
Bu _n Sn ⁽⁴⁻ⁿ⁾⁺	mussel algae	HAc _{conc.} ; overnight stirring / 30 min ultrason	NaBH ₄	GC - QFAAS	1.0 - 1.8	72
Bu _n Sn ⁽⁴⁻ⁿ⁾⁺ Ph _n Sn ⁽⁴⁻ⁿ⁾⁺	mussel fish	HCl pH 2	diethylether / 0.25 % tropolone; EtMgCl	GC - FPD	9 - 23	28
Bu _n Sn ⁽⁴⁻ⁿ⁾⁺	fish	HCl _{conc.} 4 h shaking	pentane / 0.05 % tropolone; PeMgBr	GC - FPD	40	38
Bu _n Sn ⁽⁴⁻ⁿ⁾⁺ Ph ₃ Sn ⁺	mussel fish	4 h enzymatic hydrolysis or 4 h TMAH solubilization	hexane NaBEt ₄	GC - MIP AES	2	75
Bu _n Sn ⁽⁴⁻ⁿ⁾⁺ Ph _n Sn ⁽⁴⁻ⁿ⁾⁺ Cyclohexyltins Octyltins	wine beers	enzymatic hydrolysis (pepsin, protease) ascorbic acid	HCl, tropolone pentane; MeMgCl	QF - AAS	0.04 - 0.13 ng.ml ⁻¹	65, 66
Bu _n Sn ⁽⁴⁻ⁿ⁾⁺ Ph _n Sn ⁽⁴⁻ⁿ⁾⁺ Cyclohexyltins Octyltins	fruit juices	/	HCl, tropolone pentane/diethylether MeMgCl	QF - AAS	0.03 - 0.05 ng.ml ⁻¹	67
Bu _n Sn ⁽⁴⁻ⁿ⁾⁺	urine	diethylether, tropolone hexane	NaBH ₄	QF - AAS	1 - 2 ng.ml ⁻¹	74
Bu _n Sn ⁽⁴⁻ⁿ⁾⁺	oyster	drying, homogenising HCl, HNO ₃ , or CH ₃ COOH	NaBH ₄ Purge and Trap	QF - AAS	n.g.	73
Ph _n Sn ⁽⁴⁻ⁿ⁾⁺	potatoes celery	maceration with hexane	hexane; MeMgCl	GC - FPD	2	68
Bu ₃ Sn ⁺ , Ph ₃ Sn ⁺	fish	hydrolysis, with KOH/ethanol	toluene, PrMgCl	GC - FPD	5 - 10	69
Bu _n Sn ⁽⁴⁻ⁿ⁾⁺ Me _n Sn ⁽⁴⁻ⁿ⁾⁺ R _n Sn	mussel oyster fish	24 h enzymatic hydrolysis (lipase, protease)	dichloromethane / hexane / tropolone / dithizone MeMgCl or BuMgCl	GC - QFAAS	0.2 - 0.8	25

15.5 Accuracy of the analysis

15.5.1 Preparation and quantification of calibrants

Derivatized organotin calibrants with a reasonable purity are not commercially available and must therefore be synthesized in one's own laboratory. Their use as calibrants for environmental studies necessitates an assessment of the purity of the synthesized product. They were individually prepared in octane by reacting the organotin halide with an excess of PeMgBr in diethylether (5 ml of a 2 mol.l⁻¹ solution). The reaction mixture was gently swirled around for 10 minutes at room temperature and subsequently treated with 15 ml of a 0.5 mol.l⁻¹ H_2SO_4 solution to destroy the excess of Grignard reagent. After rinsing the organic layer (2 times) with 30 ml of deionized water, a small stream of nitrogen was blown through the solution for 20 minutes to remove the excess of diethylether. Finally, the octane solution was transferred to a 25 ml volume flask and the original funnel was rinsed two times with 5 ml of octane, which joined the earlier octane solution. Additionally, octane was added to make up the volume to 25 ml. The pentylated calibrants in octane were stored in a refrigerator at 4 °C. Since, some of the alkyltin salts are not pure, the exact concentration of the stock solution is still not known. Each calibrant may contain other alkyltin compounds (e.g. degradation products) and some inorganic tin species as impurities. Therefore the first step in the quantification was the determination of the proportion between the different organotin compounds within every standard stock solution. The purity of the prepared pentylated organotin calibrants was verified by GC-QFAAS and GC mass spectrometry ion trap detection (MS-ITD). The next step in the quantification procedure is the determination of the "total" tin content of the basic stock solutions, by means of a wet acid destruction (H_2SO_4 , HNO_3 , H_2O_2) of a small fraction of the stock calibrant in an erlenmeyer fitted with a condensor [47]. All the solutions obtained after digestion were measured on the same day of destruction with flame AAS. The pentylated organotin calibrants in octane are now fully quantified, with a knowledge of the amount of inorganic tin present in the solution and the fraction of the different species. By suitable dilution in octane or hexane, mixed working calibrants were prepared for the GC-AAS and GC-AED calibration. Throughout the whole study no degradation of the pentylated calibrants was observed; even the dilute mixed working calibrant remained stable for at least 3 months if stored in a well-closed glass volumetric flask in the refrigerator and opened only shortly during use. In a similar way individual aqueous stock standards were obtained. Figure 13 shows the strategy followed for the preparation and quantification of organotin standards.

15.5.2 Interferences

The source of interferences may be due to the sample itself and/or the reagents introduced during the sample preparation procedure. The first type of interferences includes high boiling hydrocarbons which are abundant in "dirty" samples (rich in organic material). These compounds usually do not reach the detector but remain in the injection liner or in the initial part of the column and move up the column very slowly. Their presence may result in memory effects, signal discrimination as well as peak tailing and broadening. Exchangeable retention gaps between the column and the injector are recommended for effective coping with these interferences. Organic matter may be preconcentrated in the injector together with the analyte when an in-liner preconcentration system is used. Very often a dilution and/or clean-up procedure is recommended [47] for rich-in-organic matter samples.

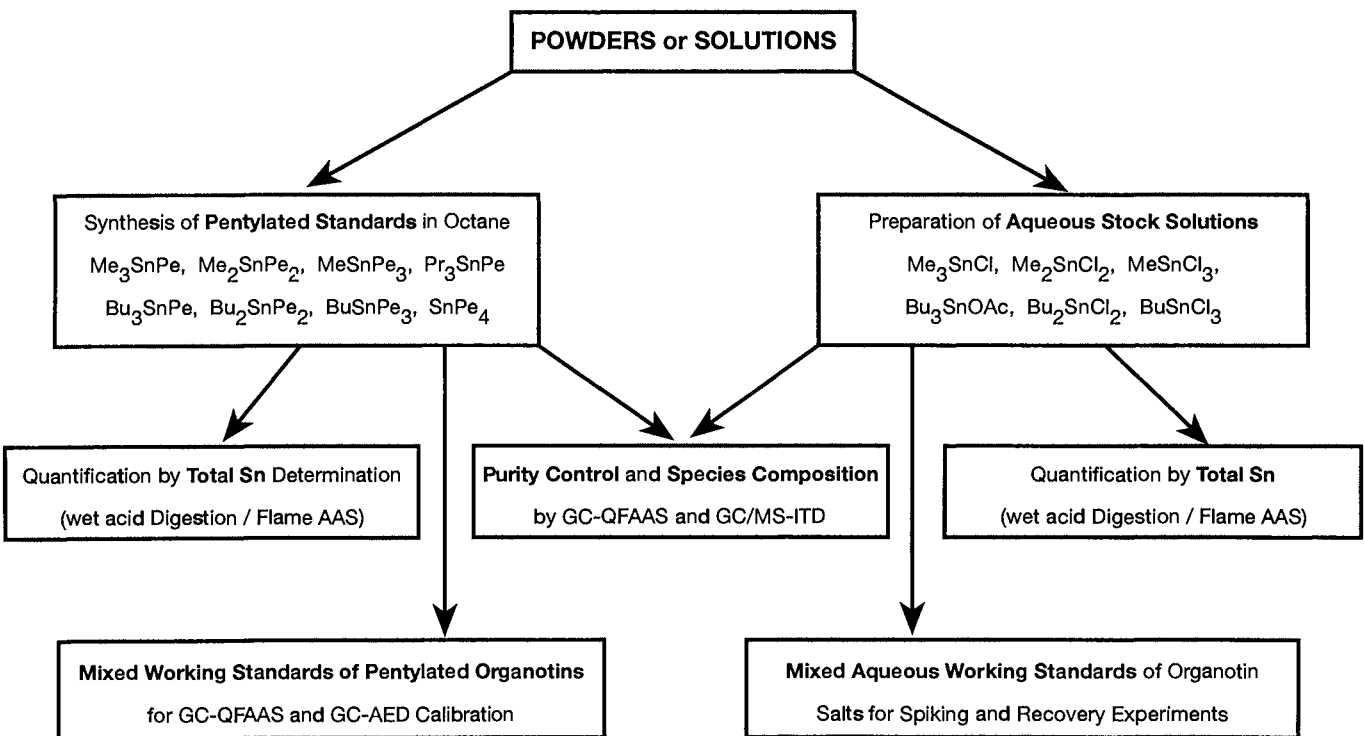


Figure 13: Scheme followed for the preparation and quantification of organotin calibrants

The second type of interferences is due to reagents used throughout the sample preparation sequence. They include the buffer and the chelating agent, sodium diethyldithiocarbamate (NaDDTC), used to assist the extraction of organotin species into pentane and finally the derivatizing agent itself. The relatively low pH value during the extraction (pH 5) [13,37,45] may be responsible for a partly decomposition of the NaDDTC and the formation of extractable products into the organic phase which are preconcentrated by rotary evaporation. The derivatization reaction may be affected under these circumstances. Further the decomposition of the Grignard reagent results in organic components (mostly impurities introduced during the synthesis of the Grignard reagent) which are extracted into the organic phase. Therefore the injected extract may contain a relatively large concentration of organic compounds.

The determination of organotin compounds in Milli-Q water spiked with ionic organotin species by GC-QFAAS did not reveal any difficulties, while signal depression and increased baseline noise appeared with the use of the graphite furnace technique and GC-AES, respectively. In the latter case blank contribution was observed during the splitless analysis and especially when large volumes were injected into the system in the in-liner preconcentration mode.

During the GC-ETAAS analysis of Milli-Q water spiked with organotins it was observed that for some species serious losses in response occurred when compared to GC-QFAAS analysis. The signals measured for MeSnPe_3 , Bu_2SnPe_2 and Bu_3SnPe constituted only 25, 80 and 75 % of the total, respectively. Decreasing the DDTC concentration in the aqueous phase by a factor of four resulted in the increase of the corresponding signals to 65, 95 and 86 % for MeSnPe_3 , Bu_2SnPe_2 and Bu_3SnPe , respectively. The use of D_2 -background correction did not result in an increase in the signals. We suspect that the signal depression is due to the elution of an unidentified compound which modifies the surface of the graphite tube. Washing the organic phase with alkaline agents (ammonia or NaOH) solved the problem only partly leading to the conclusion that it is not the DDTC which interferes but some products of its decomposition and/or the subsequent reaction of these products with the Grignard reagent.

In the case of GC-AED a more detailed study was possible due to a better sensitivity and resolving power of the system. It was observed that for split analysis the results of the recovery of the spikes agreed with those obtained by GC-QFAAS ($\pm 5\%$) while for splitless analysis signal suppression was observed. Figure 14 A, B and C shows chromatograms of a sample prepared by derivatization of hexane using PeMgCl followed by the decomposition of the Grignard reagent for $1\ \mu\text{l}$ injection and the same sample chromatographed after a 20 times in-liner preconcentration. While for the $1\ \mu\text{l}$ injection only slight correction for the contamination of the Grignard reagent is necessary, the injection of larger volumes did not result in increase in the detection capabilities due to very high blank contribution and the baseline rich in unknown peaks. In addition, the use of DDTC in the procedure hampers sensitive and reliable analyses when more than $1\ \mu\text{l}$ of the sample is injected (Figure 14 D). More research is needed to reduce the blank value of the procedure. Very low detection limits reached with GC-AES require a completely different approach in the sample preparation. *In-situ* ethylation using sodium tetraethylborate could be a viable approach [22,62].

No interferences were experienced with the AAS systems due to the lower sensitivity of this detector.

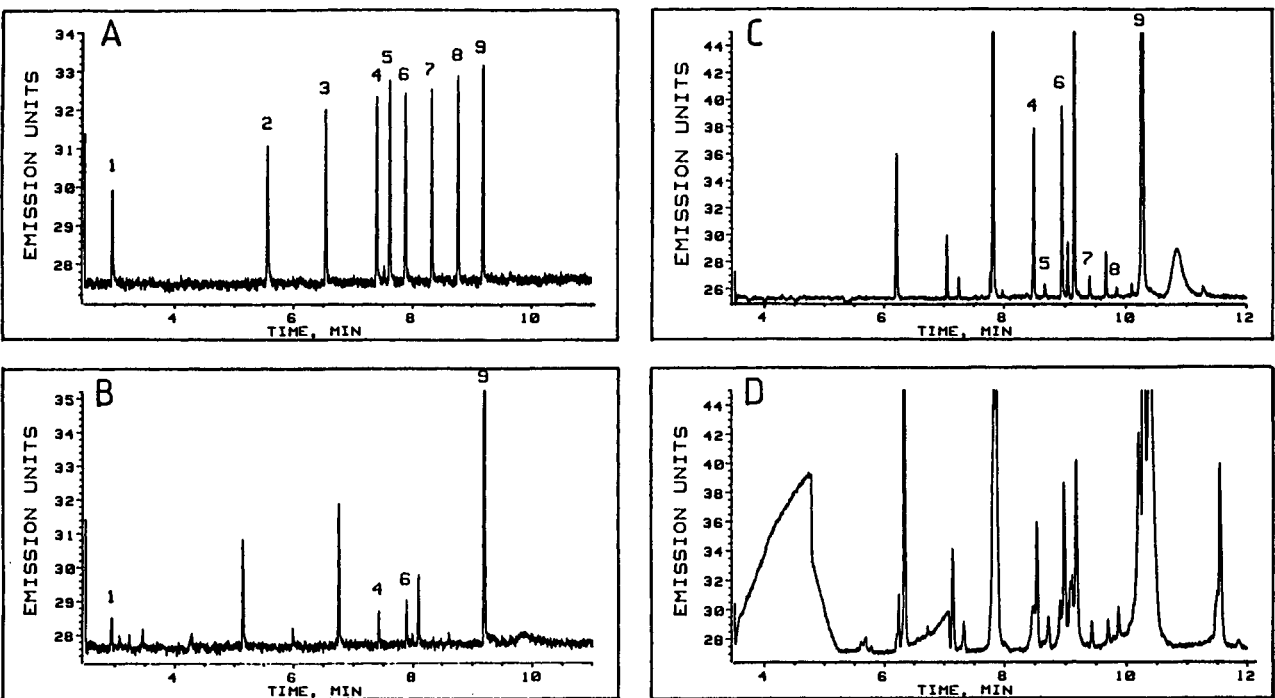


Figure 14:

GC-AES chromatograms for:

A - Mixed calibrants, 1 μ L injectionB - Hexane derivatized by n-pentyl Grignard reagent; 1 μ L injectionC - Hexane derivatized by n-pentyl Grignard reagent; 20 μ L injectionD - the blank run; 20 μ L injection

GC-AES chromatograms for:

A - Mixed standards, 1 μ L injectionB - Hexane derivatized by n-pentyl Grignard reagent; 1 μ L injectionC - Hexane derivatized by n-pentyl Grignard reagent; 20 μ L injectionD - The blank run; 20 μ L injection

15.5.3 Sources of errors

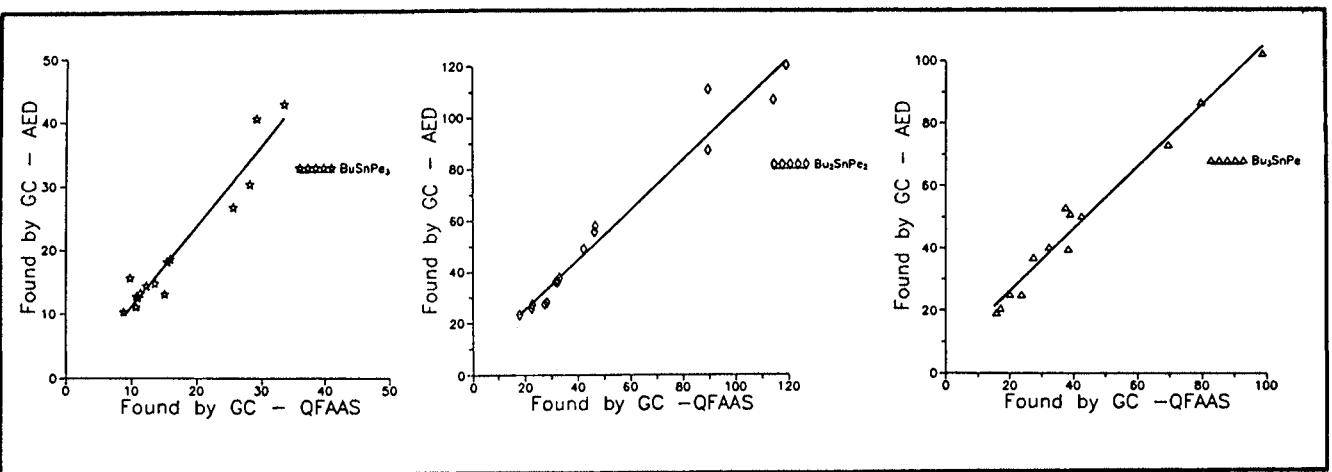
Possible errors arising during sampling and storage were recently reviewed [79-81]. The most important problems in speciation analysis for organotin compounds are apparently the adsorption onto and the release of some organotin species from certain sample-container materials (e.g. Polyvinyl chloride) and the poor recoveries of the analytes. The use of PVC [82] and polyethylene [83] was discouraged while polycarbonate and glass were found to be suitable materials [84]. Sediment samples are to a lesser degree subject to changes in concentration during storage than water samples.

While spikes on pure water are usually recovered quantitatively, an increase in particulate load can make the recoveries lower and irreproducible which, in particular, are observed during sediment analysis. Individual organotin compounds may bind to components present in a sediment to a degree varying with the salinity and the amount of particulate matter present in the water column [85] which may make the method valid for one sediment ineffective for another [31]. Therefore the determination of an extraction efficiency by spiking and extracting the spiked sediment cannot be considered reliable as one is never sure whether the species added to a sediment are adsorbed in the same way as the species initially present. It is also inadvisable to use tripropyl- or triphenyltin as an internal standard to correct for poor recoveries as the recovery usually varies for different organotin compounds in different samples.

Zhang *et al.* examined the recovery of butyltin compounds from the PACS-1 reference sediment in 10 different procedures [31]. Quantitative yields could be obtained only for TBT and DBT in a few procedures and even then signal depression problems may lead to erratic results. The method tailored for one specific determination technique often fails when another detection system is used [31]. Siu *et al.* [86] observed a 50 % reduction of signal height for the TBT spikes on the PACS-1 sediment. This was also confirmed in our laboratory but only when GC-QFAAS was used for analysis. If the same sample was analyzed by GC-AED, no suppression was noticed and the TBT recovery was quantitative [47].

15.5.4 Comparison studies

The accuracy of the techniques developed in our laboratory for organotin speciation was studied. A series of water samples from the Antwerp harbour were analyzed by GC - QFAAS and GC-AES. The extract obtained and derivatized according to the procedure, was divided into two parts and each of them was analyzed by a different technique to evaluate the accuracy of the detection. Results are shown in Figures 15 A - C for BuSnPe_3 , Bu_2SnPe_2 and Bu_3SnPe . The results agreed within 10 %. It must be emphasized, however, that this good agreement was obtained only in the split mode but at the expense of sensitivity. When the samples were run in the splitless mode, higher results (by about 20 %) were obtained with AES detection.



Comparison of the results obtained by GC-AES and GC-QFAAS detection for the analysis of harbour water.

A - BuSn^{3+}

B - $\text{Bu}_2\text{Sn}^{2+}$

C - Bu_3Sn^+

Figure 15: Comparison of the results obtained by GC-AES and GC-QFAAS detection for the analysis of harbour water.
A - BuSnPe_3 ; B - Bu_2SnPe_2 ; C - Bu_3SnPe

15.5.5 Interlaboratory studies

The methodology for organotin speciation analysis continues to be the subject of intense development. Many hyphenated techniques are currently in use and the number of environmental data published is constantly growing. The evaluation of the analytical quality of these data is the most important issue at the moment. The most practical way to control the accuracy and precision of analytical results is through the use of appropriate Certified Reference Materials (CRMs) [87]. Both the National Institute of Standards and Technology (NIST, USA) and the Measurement and Testing Programme of the EC, formerly Community Bureau of Reference (BCR) have initiated interlaboratory studies aiming at issuing certified reference materials for organotins.

A certified reference material for TBT, DBT and MBT in sediment (marine harbour sediment (PACS-1)) has been prepared by the National Research Council of Canada with concentrations of 1.21 ± 0.24 for TBT; 1.14 ± 0.20 for DBT and 0.28 ± 0.17 for MBT ($\mu\text{g.g}^{-1}$ as Sn, in dry sample).

The BCR recently certified a reference material for TBT and DBT in an estuarine sediment (CRM 462) with contents of 70.5 ± 13.2 for TBT and 128 ± 16 for DBT (ng.g^{-1} as cation, in dry sample), which concentration levels are much lower than the earlier mentioned PACS-1. Another candidate material (harbour sediment, CRM 424) is still under investigation and will be certified as soon as the results obtained so far are confirmed by a sufficient number of independent methods [88].

The National Institute for Environmental Studies (NIES, Japan) issued a CRM 11, which is a fish tissue with a certified content of TBT of $1.3 \pm 0.1 \mu\text{g.g}^{-1}$ and a reference value ($6.3 \mu\text{g.g}^{-1}$) for TPhT (both values as chlorides). No CRM is so far available for water samples.

15.6 Conclusions

Gas chromatography with atomic spectrometric detection offers an unrivalled combination of sensitivity and selectivity for the speciation analysis of organotin. At present, quartz furnace atomization coupled to megabore column separation is the most recommended approach in AAS speciation analysis of organotin, due to relatively easy availability, low cost of operation and low vulnerability to interferences. A careful design of the interface and optimization of the operating variables is necessary. The use of capillary columns offering excellent resolution is still hampered by low column capacity but the rapid development of large volume injection techniques is expected to provide viable approaches in the near future. A wider availability of the commercial gas chromatograph - atomic emission spectrometer is likely to bring the speciation analysis towards lower concentration levels. Also, could it simplify the now complicated sample preparation sequences enabling unattended routine analysis. The development of effective, interference free and easy to be automated sample preparation procedures is a concern at this moment. The development of other procedures independent of hydride generation is also likely to project more light on the problem of accuracy in the speciation analysis of organotin at low ng.l^{-1} levels.

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16.

High performance liquid chromatography - isotope dilution - inductively coupled plasma - mass spectrometry for lead and tin speciation in environmental samples

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It is well known that the mobility, toxicity and fate of metals in the environment depends critically on the form of the metal. The presence of both organolead and organotin compounds are currently of particular concern owing to large scale anthropogenic inputs. In addition, it has been suggested that both organolead and organotin compounds are also derived from natural biotic and abiotic methylation processes [1]. Many reports now exist describing the effects of specific lead and tin compounds on the environment. For example, tetraalkyllead compounds (used as antiknock compounds in petrol) and their degradation products the tri- and dialkylleads, have been reported in various environmental samples including waters, soils, fish and vegetation [2,3]. Organotin compounds have also been widely reported to be present in environmental samples. Tributyltin in particular has been the subject of many studies and there is now a considerable literature detailing the presence, fate, degradation and biological effects of the compound. Additional information on organo-lead and tin speciation can be found in Chapters 14 and 15 of this book.

To evaluate the environmental impact of lead and tin and to study environmental pathways there is a obviously a clear need for versatile yet sensitive elemental techniques to both separate and quantify individual species, and for reference materials to help in the validation of such techniques. This paper describes work undertaken as part of the Measurements and Testing Programme (BCR) to provide valid reference materials containing organolead and organotin species.

16.1 Overview of speciation methods for lead and tin

In order to speciate organolead and organotin compounds it is usually necessary to first separate the organometallic species, usually by chromatography, and then to determine the individual species using a sensitive detector. Atomic spectroscopy is the most common technique employed for detection since it is element specific and offers the sensitivity required. This technique has been described in detail in Chapters 14 and 15 of this book. The chromatography needs only separate the metal containing species of interest since other coeluting compounds will not be detected. Other analytical techniques do exist for the determination of organometallic species. For example the total alkyllead content of a sample may be determined using a dithizone extraction followed by acidic back-extraction and atomic absorption measurement. However such techniques do not give data on individual species and so are of limited use in rigorous speciation studies.

There are many approaches to metal speciation using coupled chromatography -atomic spectroscopy [4-6]. The couplings most often employed are summarised in Table 1. It is convenient to group these techniques according to the nature of the chromatographic separation employed. Both organolead and organotin compounds may be separated using gas chromatography (GC). This approach has been used coupled to atomic absorption spectrometry (AAS); various optical emission techniques including microwave induced plasmas (MIP), direct current plasmas (DCP), and inductively coupled plasmas (ICP); and atomic fluorescence spectrometers (AFS). To date the speciation of lead in petrol by GC-AAS is the most widely reported use of a coupled technique for speciation reflecting the high sensitivity and selectivity of the technique and the minimal sample handling required for this application. This last point is important since the ideal technique should involve the minimum of sample pretreatment and preconcentration in order to ensure that the integrity of the original sample remains intact. Unfortunately many organolead and organotin species require derivatisation, often methylation or butylation, prior to their separation by GC. However many groups of workers have successfully used GC techniques to good effect and have reported absolute detection limits for both organolead and organotin species in the low pg range (as Pb).

The alternative approach to using gas chromatography for the separation is the use of high performance liquid chromatography (HPLC). Again atomic spectroscopy has been widely used for detection as summarised in Table 1. Although the couplings required for HPLC systems tend to require more thought, there is no need for sample derivatisation and many applications of coupled HPLC systems for the determination of organometallics have been reported [5,6]. Until recently the major limitation of the use of coupled HPLC techniques has been the disappointing detection limits obtained. However with the increasing availability of ICP-MS instruments in many laboratories to act as the detection system, significantly lower detection limits are now being reported [6]. In addition the use of ICP-MS also offers simultaneous multi-element capabilities and the facility for isotope dilution studies .

Gas Chromatography.	High performance liquid chromatography.
Flame AAS. Electrically heated furnace AAS. Graphite furnace AAS. Microwave plasma - AES. Direct current plasma - AES. Inductively coupled plasma -AES. Atomic fluorescence spectrometry. Inductively coupled plasma - mass spectrometry. Also: As above incorporating hydride generation stage. As above incorporating preconcentration step e.g. cryogenic trapping.	Flame AAS. Graphite Furnace AAS. Microwave plasma - AES. Direct current plasma - AES. Inductively coupled plasma - AES. Atomic fluorescence spectrometry. Inductively coupled plasma - mass spectrometry. Also: As above incorporating an hydride generation stage. Various novel sample transport devices e.g. rotating spirals (Ref [7]).

TABLE 1: Summary of coupled gas chromatography - atomic spectrometry and high performance liquid chromatography - atomic spectrometry techniques available for metal speciation.

16.2 Isotope Dilution in HPLC-ICP-MS

One of the many advantages of ICP-MS apart from its inherent sensitivity (generally 2-3 orders of magnitude better than ICP-AES) is the possibility of measuring isotope ratios (IR) due to the MS component in the technique. "Chemical Abstracts" list over 130 papers that included ICP-MS and IR in the studies described over the past 10 years. A logical extension to isotope ratio measurement is to incorporate the procedure into an isotope dilution (ID) calibration strategy. The main advantages of isotope dilution analysis (IDA) are increased accuracy and precision. The increase in accuracy can be ascribed to the fact that IDA is essentially a standard additions technique using one isotope of the analyte as its own internal standard. Further, if the isotopic spike is added prior to any sample extraction or digestion then any losses during digestion or incomplete extraction of analyte can be taken into account. The improved precision can be attributed to the elimination of short term drift including instrument drift and nebuliser drift since an isotope ratio is being measured and because noise tends to cancel out. Typically for lead, the single measurement of m/z 206 or 208 may produce precisions (RSD%) of 0.5-1.0% however, the isotope ratio 206/208 would produce precisions of 0.1-0.2% (12). The subject of IDA has been extensively reviewed in the Chapter 2 of this book.

In our work we have extended the principle of IDA to HPLC-ICP-MS. Again, this could be considered a logical extension of the method to achieve improved accuracy. While about 50 papers have been published in recent years on coupled HPLC-ICP-MS none published before 1993 report ID-HPLC-ICP-MS. As discussed later, the reason for this is probably due to the fact that there are many practical problems that need to be addressed and overcome.

Whilst the expectation of the IDA technique is to improve accuracy and precision however, in ID-HPLC-ICP-MS coupled methods there are also other considerations. Firstly, accuracy of any analytical method depends upon a traceable calibrant. In our HPLC-ICP-MS application, organometallic species are being determined. Not only are the relevant organometallic compounds required for calibrants, they are required to be enriched in one of the analyte isotopes. In general, these are not commercially available and hence it is necessary to synthesise them and determine purity. In ID-HPLC-ICP-MS therefore, accuracy is more a function of calibrant availability and purity, which is generally not considered a major problem in ID-ICP-MS and generally, in analytical chemistry nowadays. Secondly, in conventional ID-ICP-MS the final precision of the result is mainly dependant upon the precision of the isotope ratio measurement. As mentioned earlier, precisions (RSD %) for lead IR measurements of 0.1-0.2% have been obtained and this is on a routine basis. However, in an ID-HPLC-ICP-MS method, the limiting precision may well be that of the reproducibility of manual sample injection onto a HPLC column. Generally, a precision of 2-4 % is normally associated with this.

The following sections are concerned mainly with these practical considerations and problems encountered in our method development work for principally lead, and also tin speciation using ID-HPLC-ICP-MS.

16.3 Practical considerations in ID-HPLC-ICP-MS

In general, metal speciation is obtained by the coupling of a separation technique to an element specific detector. For the HPLC-ICP-MS coupled system in question this, on its own, can lead to problems of peak broadening and hence loss of resolution in the final chromatogram due to dead volume within the coupling. When ID is incorporated into the procedure in order to attempt to improve accuracy and precision, further practical problems are encountered. Although software is available for ID-ICP-MS, and it is also possible on some commercial ICP-MS instruments to perform HPLC-ICP-MS, there is no commercially-available customised software for ID-HPLC-ICP-MS. In our work on HPLC-ICP-MS, for lead especially, a further consideration was contamination from lead in the buffer solution and lead leached from the HPLC pump. Although small in concentration (1-2 ng.ml⁻¹) this proved to be a problem when gradient elution chromatography was used due to sensitivity differences for lead in increasing amounts of methanol.

16.3.1 Coupling HPLC to ICP-MS

When HPLC is coupled to ICP-AES or ICP-MS the simplest coupling is to connect the eluent from the column directly to the nebuliser via a piece of plastic tubing. A flow rate of 1 ml min⁻¹ is fully compatible for both HPLC separations and ICP sample introduction. It has been known for many years that the transfer capillary tubing should be as narrow as possible and as short as possible to avoid broadening effects. These precautions reduce dead volume in the liquid flowing stream. A tube diameter of 0.2 mm is now

used in our laboratory. A further consideration is that of dead volume in the vapour or gas phase. The conventional system for sample introduction into a ICP-MS instrument is the standard nebuliser/spray chamber assembly, in our case a Scott double-pass spray chamber with a concentric glass nebuliser or 'V'-groove nebuliser. Figure 1A shows a peak obtained by HPLC-ICP-MS for As using the conventional Scott spray chamber design (120 mls) [9]. Figure 1B shows the same separation but using a mini-spray chamber based on the design of Kempster *et al.* [10] which had an internal volume of 11 ml. It is quite clear that the peak in Figure 1B shows a narrower peak and this indicates that gas phase dead volume within the spray chamber can produce peak broadening effects. There are various ways to improve the situation, however, in all cases a compromise must be reached. In general, a small volume spray chamber would be better than a large volume spray chamber to retain chromatographic resolution. However, small volume spray chambers are less efficient and cause larger droplets to reach the plasma causing noise. Further, large volume spray chambers tend to dampen pulses from, for example, the pumping system and therefore the smaller the volume, the larger the disturbances from pump pulsing and hence noise. An alternative approach would be to omit the spray chamber completely and pass the eluent from the HPLC column directly into the plasma using direct injection nebulisation (DIN)[11]. Such devices have been constructed and evaluated for HPLC-ICP-MS and show some promise but are, however, relatively expensive and in our experience fragile. Problems with pulsing are likely to be exacerbated. They also tend to favour more microbore column HPLC and low mobile phase flow rates.

In the lead speciation studies using HPLC-ICP-MS, the main species under investigation were the inorganic lead, trimethyl lead and triethyl lead ions. The tetraalkyllead compounds are easily separated by GC unlike the trialkyllead compounds that require derivatisation (usually butylation) prior to GC which is time consuming [12,13]. For the initial studies to investigate the separation of these species by HPLC, an atomic absorption spectrometer was used as the metal specific detector since the performance of this detector is well characterised [12]. The eluent from the column was connected directly to the nebuliser by a short length of capillary tubing. It is important to note that the rise time of the sample liquid in an AAS spray chamber is about 6 seconds, i.e. it takes that time for a solution to pass through the spray chamber to the flame to give a stable signal. So, although the volume of the spray chamber is relatively high (100 ml) the transport velocity of the aerosol through the spray chamber is also high (10 l.min⁻¹).

The chromatographic starting conditions were essentially those described by Al-Rashdan *et al* [13,14]. The separation is based on reversed phase HPLC using an acetate (0.1 mol.l⁻¹): methanol mobile phase and sodium pentane sulfonate (4 mmol.l⁻¹) as an ion-pairing reagent. The initial studies were performed using different mixtures of the aqueous buffer solution (0.1 mol.l⁻¹ of sodium acetate and acetic acid, pH 4.6 and 4 mmol.l⁻¹ sodium pentane sulfonate) and methanol to obtain the most suitable conditions for a separation under isocratic chromatographic conditions. Test solutions contained mixtures of Pb²⁺ and Me₃Pb⁺ at concentrations of 50-100 mg.l⁻¹. The results can be summarised below:

- (i) a 70:30 methanol:buffer mixture produced only one peak close to the solvent front;
- (ii) a 50:50 methanol:buffer mixture resulted in two unresolved peaks, the leading peak being inorganic Pb which was close to the solvent front;
- (iii) a 30:70 methanol:buffer mixture produced two baseline-resolved peaks in 10 minutes but with the Me_3Pb^+ peak showing substantial tailing;
- (iv) a better separation was obtained using a 40:60 mixture as shown in Figure 2A.

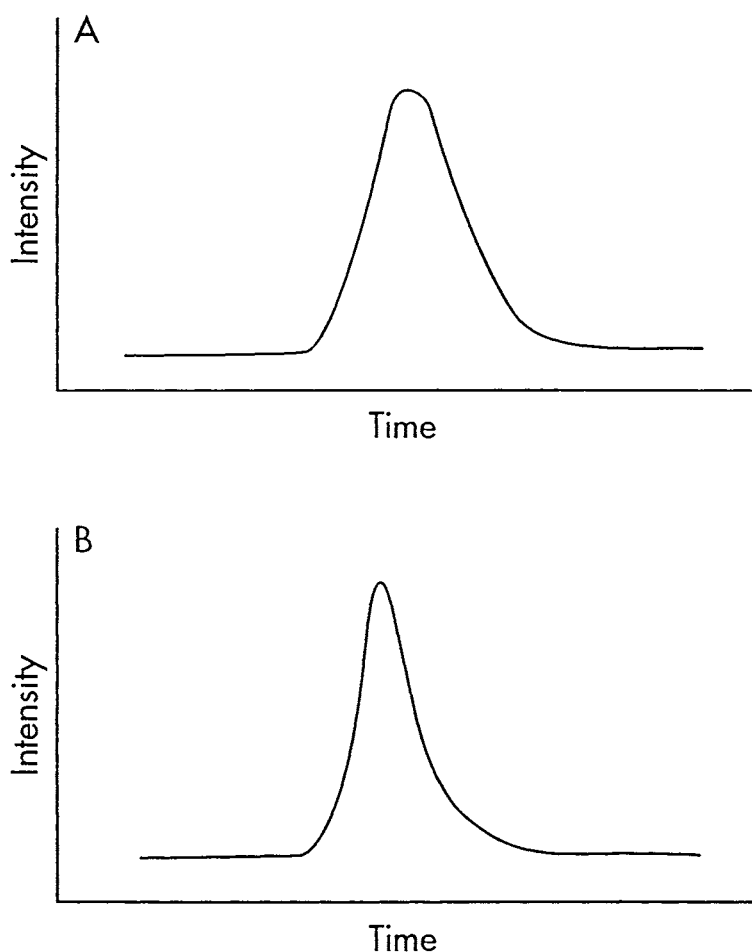


Figure 1: Chromatographic peaks (following smoothing to remove noise) for arsenic using HPLC-ICP-MS. A- Scott double-pass spray chamber, B- mini spray chamber

The result of adding Et_3Pb^+ into the separation using a 60:40 methanol:buffer mixture is shown in Figure 2B. At lower methanol concentrations it was found that the Et_3Pb^+ took longer to elute and at higher concentrations merged with the Me_3Pb^+ . Thus, at this stage the chromatography was 'characterised' for Pb^{2+} , Me_3Pb^+ and Et_3Pb^+ using the AAS detector system. The optimum conditions for the chromatography indicated using a 40:60 methanol:buffer mixture for the Pb^{2+} and Me_3Pb^+ separation, and then a step to 60:40 to elute the Et_3Pb^+ .

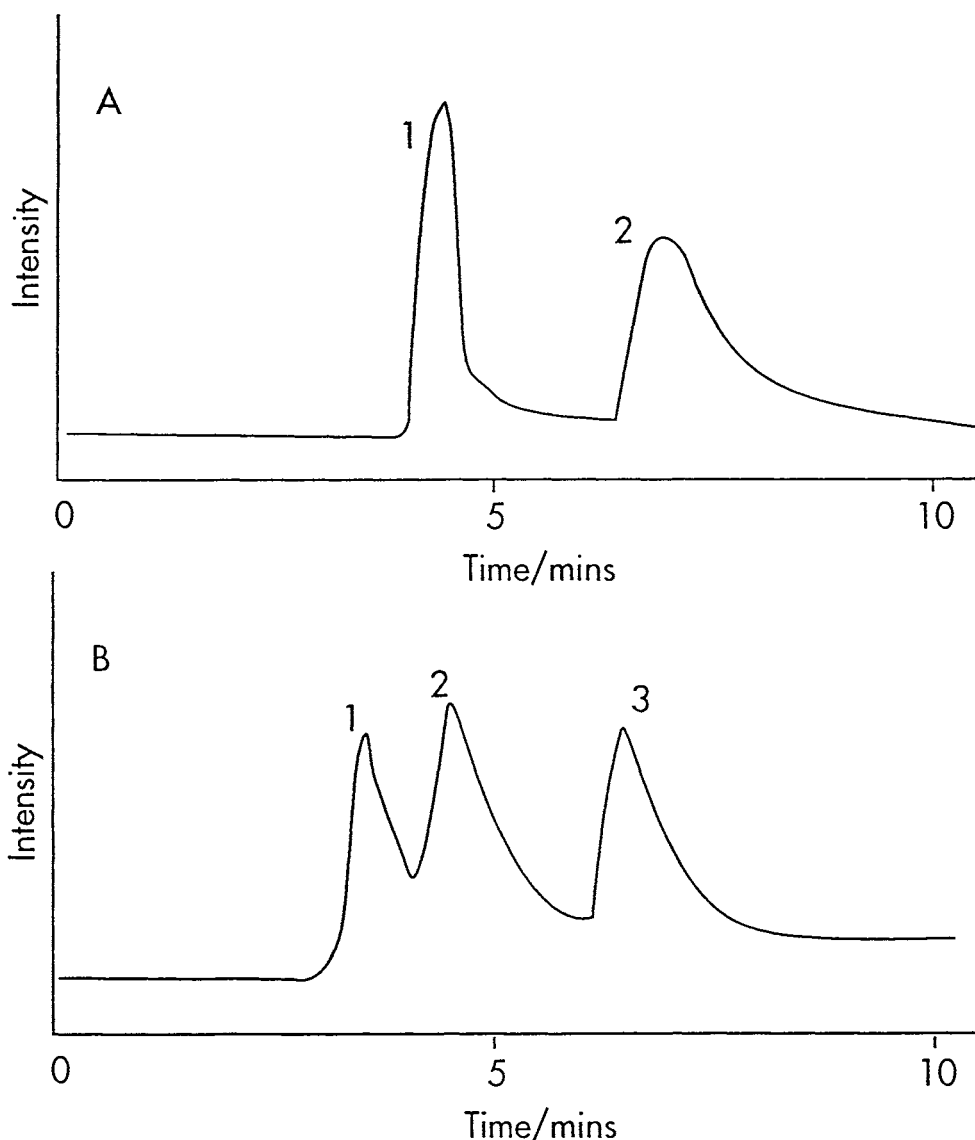


Figure 2: HPLC-AAS chromatograms using isocratic conditions of A- 40:60 ratio of methanol to buffer and B- 60:40 ratio of methanol to buffer. Peaks 1, 2 and 3 correspond to Pb^{2+} , Me_3Pb^+ and Et_3Pb^+ respectively

The expectation was that the chromatographic separation developed for HPLC-AAS would transfer, with only minor modification, to HPLC-ICP-MS. The very first attempt at achieving a separation of Pb^{2+} and Me_3Pb^+ is shown in Figure 3A. In this case the eluent was connected directly to a high solids 'V' groove nebuliser fitted to a standard Scott double-pass glass spray chamber. The mobile phase was a 40:60 methanol:buffer mixture which had been shown previously to work well with the flame AAS coupling using a flow rate of $1 \text{ ml} \cdot \text{min}^{-1}$. It can be seen that baseline resolution had been lost. In order to obtain baseline resolution under isocratic conditions the methanol concentration was systematically reduced to a 10:90 methanol:buffer mobile phase. Under these conditions baseline resolution was achieved, however, the Me_3Pb^+ peak took up to 40 minutes to elute fully and was 15 minutes wide showing excessive tailing. The separation was not suitable for quantisation purposes.

These results highlight the importance of dead volume in a HPLC-ICP-MS interface. At this point the complete coupling system was dismantled and examined closely. Upon inspection, the 'V'-groove nebuliser revealed a substantial dead volume between the capillary tubing end-cap and the nebuliser exit port. This was replaced with a glass concentric nebuliser (J. F. Meinhard Associates Inc, Santa Ana, CA92705, USA). The tubing from the end of the column to the nebuliser was reduced to 0.2 mm internal diameter and halved in length to 350 mm. These steps improved the situation as shown in Figure 3B. This separation was achieved with a 30:70 methanol:buffer mixture (isocratic) and $1 \text{ mg} \cdot \text{l}^{-1} \text{ Pb}^{2+}$ and $5 \text{ mg} \cdot \text{l}^{-1} \text{ Me}_3\text{Pb}^+$. The separation still shows excessive tailing of the Pb^{2+} peak and again baseline resolution was not obtained.

Attention was then focused upon the Scott double-pass spray chamber. The design of this spray chamber is such that the nebulised sample passes down a glass cylinder inside the spray chamber and then back outside the cylinder and into the plasma. For conventional sample introduction this design has long been accepted as standard. In chromatography terms the design may not be optimum since there are places in the spray chamber where samples can 'linger' (e.g. at both ends) and also the surface area of glass with which the sample comes in contact with is vast. This second point may be more important for elements that tend to 'plate out' or 'stick' on to glass surfaces especially since the carrier gas flow rate is only around $0.7 \text{ l} \cdot \text{min}^{-1}$. Between pH 4-7, lead falls into this category. Although the peak tailing observed in Figure 3B could be due to a chromatographic problem there is a higher chance that it was due to these two other explanations since this effect was not that apparent in the flame AAS coupling shown in Figure 2A.

Due to this the Scott double-pass spray chamber was replaced by a single-pass spray chamber of in-house construction which had an internal volume of 40 ml. This spray chamber had a cooling jacket and was chilled to -15°C . With the original system it was found to take 4-5 minutes for the signal to stabilise under conventional nebulisation conditions. This is significantly longer than would normally be expected, however the modified sample interface produced stable signals within 60 seconds. Although this is clearly superior to the original system, it is still 8-10 times longer than the AAS system used in the early development work. This suggests that further investigation into sample introduction systems for HPLC-ICP-MS is needed.

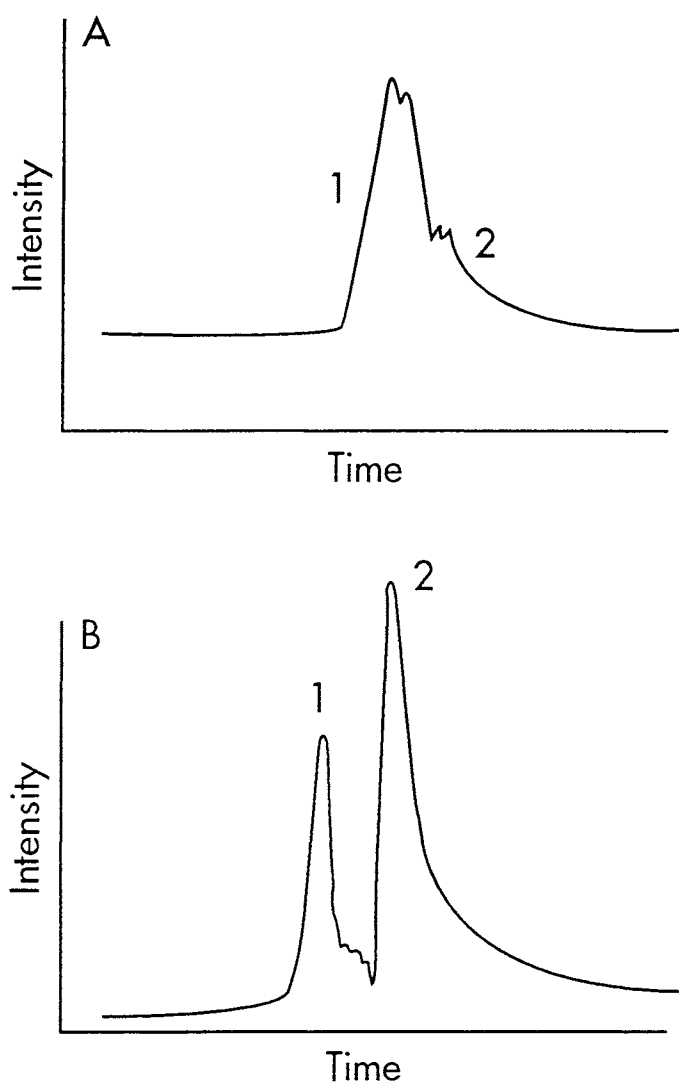


Figure 3: HPLC-ICP-MS chromatograms for 1- Pb^{2+} and 2- Me_3Pb^+ . A- isocratic 40:60 ratio of methanol to buffer. Worse-case interface with excessive dead volume. B- 30:70 ratio of methanol to buffer. Partly-optimised interface but showing no baseline resolution.

Figure 4A shows a chromatogram run under isocratic conditions with a 17.5:82.5 methanol:buffer mixture using the improved interface system. The separation of Pb^{2+} and Me_3Pb^+ took about 30 minutes and could be used for quantitative purposes although the Me_3Pb^+ peak still showed signs of tailing. These results demonstrate the improvements which can be achieved by following a systematic approach to reducing dead volume in interface systems when coupling HPLC to ICP-MS. For many separations the standard Scott spray chamber assembly may be completely adequate. However, to obtain the baseline resolution required for quantitative determination and for the separation of Me_3Pb^+ from Pb^{2+} the single-pass spray chamber produced better performance.

In order to refine the chromatography, the separation shown in Figure 4A would clearly benefit from a gradient elution system. To this end a gradient HPLC system was employed and the result of a separation of $1 \mu\text{g ml}^{-1}$ Pb^{2+} and Me_3Pb^+ is shown in Figure 4B. The separation starts with a 10:90 methanol:buffer mobile phase mixture for 2 minutes and after 10 minutes the mixture is 30:70 for a further 5 minutes. The peaks can be seen to be well resolved with a separation time of only 15 minutes. Experience with gradients in HPLC-ICP-MS is discussed in the next section.

16.3.2 Potential problems with gradient elution HPLC-ICP-MS

From the separations shown in Figure 4B it is clear that a gradient elution programme speeds up the separation and also produces a Me_3Pb^+ peak which is relatively sharp and free from peak tailing. Although this is a successful result, it could be expected that a real sample would contain a vast excess of inorganic lead compared to organolead species. It is under these conditions that lead contamination in the mobile phase buffer, associated with varying amounts of methanol due to the gradient elution chromatographic scheme, causes a varying baseline in HPLC-ICP-MS. The contribution from the blank can be seen as a baseline off-set in Figures 3A and B.

Upon investigation of the baseline off-set, the expected isotope ratio values for Pb at m/z 204, 206, 207 and 208 were found and this indicated that the off-set was due to lead contamination. The components of the mobile phase were individually analysed by ICP-MS at the concentrations used (0.1 mol.l^{-1} acetic acid and sodium acetate and 4 mmol.l^{-1} sodium pentane sulfonate). The results showed that the acetic acid contained less than $0.25 \mu\text{g.l}^{-1}$ Pb but that the sodium acetate contained $1.7 \mu\text{g.l}^{-1}$ and the sodium pentane sulfonate contained $1.9 \mu\text{g.l}^{-1}$ Pb.

New chemicals were purchased and analysed for lead. Also, the use of glass volumetric flasks was replaced by plastic containers in an attempt to minimise lead contamination. Further, a new HPLC pump (Varian 9010, Warrington, UK) was purchased to enable gradient elution chromatography. Under these conditions the lead content in the buffer was reduced to less than $1 \mu\text{g.l}^{-1}$, however, the lead signal from the eluent was still equivalent to about $4 \mu\text{g.l}^{-1}$ lead. Further investigations indicated that the lead was from the pump and in particular, from the small mixing chamber and pulse dampener in the HPLC system and not the analytical column. Work is in progress to reduce this contamination. Al-Rashdan and co-workers [15,16] also experienced the same contamination source in their work.

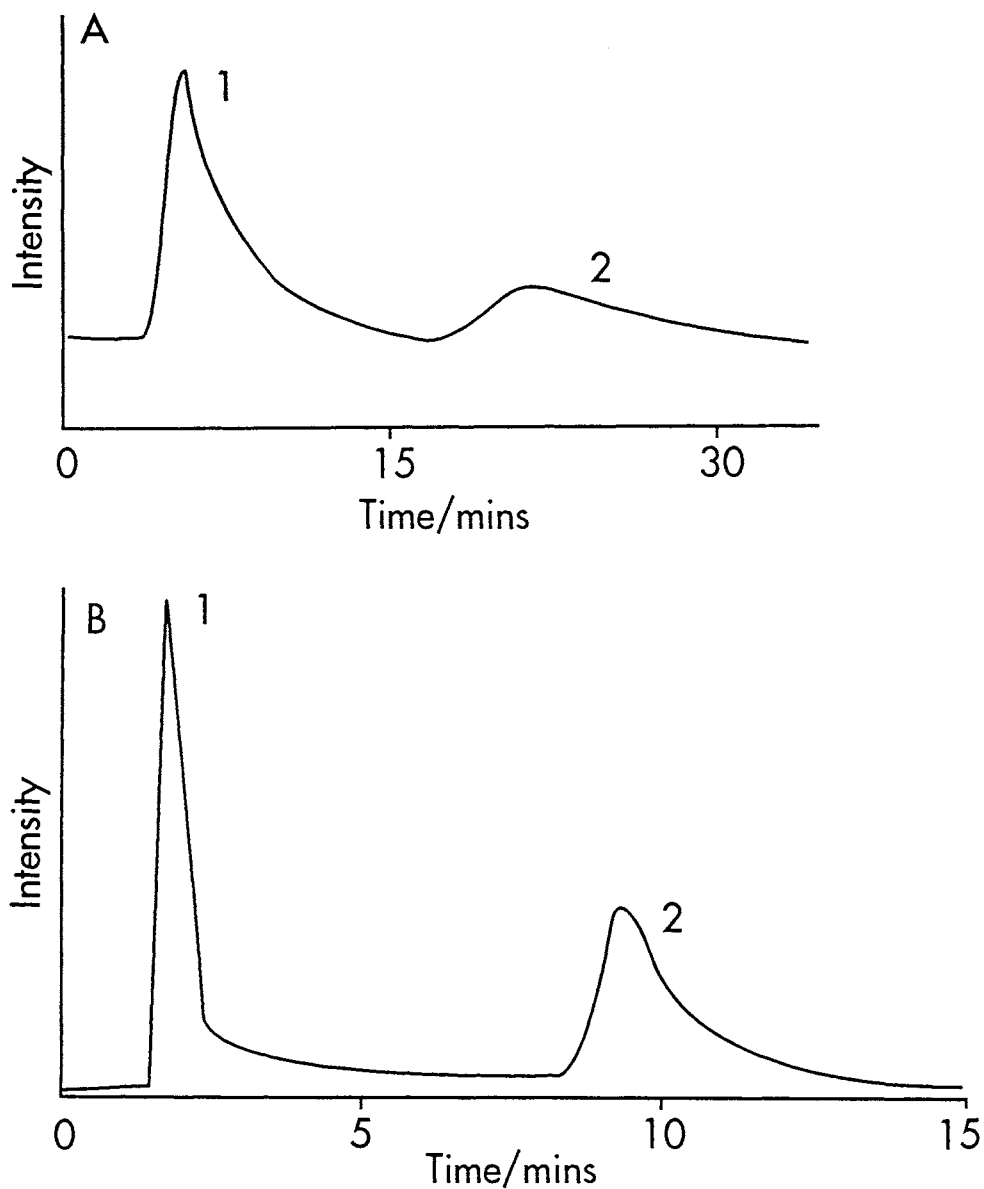


Figure 4: HPLC-ICP-MS chromatogram for 1- Pb^{2+} and 2- Me_3Pb^+ . A- isocratic 17.5-82.5 ratio of methanol to buffer, reduced volume spray chamber. B- gradient elution chromatograph with reduced volume spray chamber at 1 mg.l^{-1} concentration of analytes.

Figure 5A shows a chromatogram using only the HPLC pump to run a gradient. In this case the gradient was run as 0 to 3 minutes - 10:90 methanol to buffer ratio and 3 to 10 minutes - gradient to 30:70 methanol to buffer ratio. The total run time was 1803 seconds. There was no sample added and no valve switching involved. The ICP-MS instrument was optimised in this case with an aqueous buffer solution containing $100 \mu\text{g.l}^{-1}$ Pb and then left for about 30 minutes to wash out the spray chamber. In an isocratic chromatographic run the baseline remains constant throughout the run. In the gradient elution case (Figure 5A), the baseline remains constant (about 4,000 counts) until the start of the methanol gradient, then decreases steadily until the end of the gradient, where it remains constant at this decreased sensitivity until the end of the run.

In order to explain this, individual solutions containing different concentrations of methanol (0-30 %) and $50 \mu\text{g.l}^{-1}$ lead were investigated. The trend observed for the lead signal was that of a steady decrease in signal intensity of 0-65 % in 0-30 % methanol. In this first experiment the signal was optimised with an aqueous solution. Further experiments were performed, but in each case the lens settings on the ICP-MS instrument were optimised for 1, 10, 20 or 30% methanol solutions, and then the series of 0-30% methanol solutions run. The results showed that the effect of methanol concentration on lead intensity in ICP-MS varied depending upon the lens settings of the ICP-MS instrument. As an example, the maximum signal obtained from a solution of $50 \mu\text{g.l}^{-1}$ lead in 20 % methanol was obtained with different lens settings, especially the extraction lens, compared to an aqueous solution. A situation was reached that when the optimised conditions for the 20 % methanol solution were used to run a gradient in HPLC, the signal at the beginning of the chromatographic run was suppressed. This situation is shown in Figure 5B. In this blank gradient run, the baseline remains constant for a time then rises to a particular value, then follows the depressive response observed in the case shown in Figure 5A.

The effect of methanol on lead is dependent upon lens settings, especially the extraction lens, which tends to suggest that the methanol changes the spatial position within the plasma where maximum lead ion concentration is found. Further experiments were carried out on this phenomena and the situation is far more complex than it first appeared. The effects vary depending on position of plasma, nebuliser gas flow and lens settings and further investigations into this area of research are continuing. On one occasion, a situation was reached where the baseline was actually constant over the whole gradient run and decreased only after the Me_3Pb^+ peak had eluted. This condition was only reached after 4 hours of working, lasted only for 2 hours, and was probably reached by a component of instrument drift in the quadrupole lens stack. These conditions have proved elusive to define however, it does indicate that a set of conditions exists which can minimise gradient problems in HPLC-ICP-MS.

In HPLC terms the baseline fluctuations are due to the effect of methanol on lead present as a small contaminant in the eluent. Various ways to reduce this contamination are being actively sought such as acid washing of the pump manifold and by employing a buffer 'clean-up' stage. These steps should minimise the problem which is only apparent when measuring near the detection limit for lead (*i.e.* at parts per billion levels). At high lead concentrations the effects are masked by the decreased electronic gains employed.

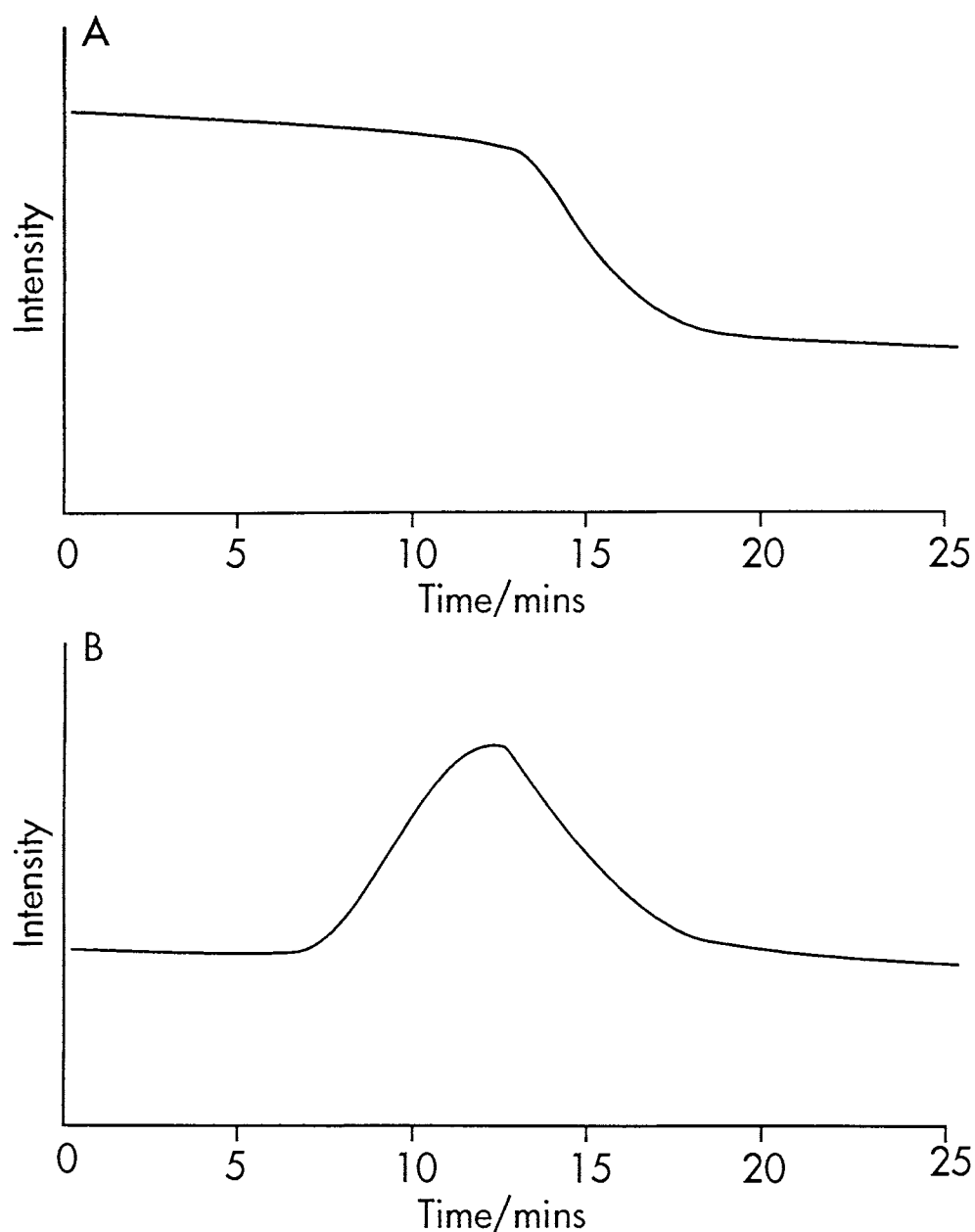


Figure 5: Effect of methanol gradient on the baseline response in HPLC-ICP-MS. A- ICP-MS optimised on an aqueous buffer containing $100 \mu\text{g.l}^{-1}$ Pb. B- ICP-MS optimised on a solution containing $100 \mu\text{g.l}^{-1}$ Pb, 20 % methanol and 80 % buffer.

16.3.3 Organometallic calibrants for isotope dilution analysis

One of the main problems associated with the ID-HPLC-ICP-MS procedure is that of calibrant availability and purity. As a general aim, the calibrants should be at least 90% pure but more importantly, their purity should be known accurately. For lead, the compounds which were of interest to the present study were the trialkyllead salts (trimethyllead chloride and triethyllead chloride). For tin, the compounds were tributyltin chloride, dibutyltin chloride and monobutyltin chloride.

Traditionally, the conventional IDA strategy has suffered from being relatively expensive. Given the growing popularity of IDA, commercial calibrants are now available from a number of sources and for many elements at reasonable prices. Isotopically enriched organometallic calibrants for ID-HPLC-ICP-MS is, however, a new requirement. Currently, no supplier exists that routinely manufactures these types of calibrants. Consequently, the standards must be synthesised from, usually, pure isotopically enriched analyte metal. This poses a potential problem to the analyst. Since most IDA methods involve a MS determination of isotopes the analysis is usually very sensitive. Therefore, the commercially available calibrants are usually in the range of 50-100 mg.l⁻¹ solutions. This keeps the price of such calibrants relatively low. When it becomes necessary to purchase gram quantities of enriched analyte to synthesise organometallic salts then the price of the calibrants escalates and even then the purity of such calibrants requires extensive investigations. Further, many of the compounds required may be synthesised via organic reactions with yields significantly less than 100%. To summarise therefore, isotopically enriched organometallic standards may prove to be the single greatest problem at present for ID-HPLC-ICP-MS simply due to cost and synthesis/purity problems. The cost disadvantage is element dependent and the synthesis problem is species dependent. It is assumed that the analyst would be able to assess purity. In time, these potential problems may decrease in importance in the same way that problems with calibrants for conventional ID-MS did.

16.3.3.1 Preparation of enriched lead isotope calibrants

The calibrants required for lead were isotopically enriched Me₃PbCl and Et₃PbCl. The choice of isotope to be used for the enrichment may well depend upon availability rather than any theoretical considerations. From theoretical considerations it is better to choose a minor isotope of the analyte which, upon addition of the spike to the sample, produces an isotope ratio value between 0.5 and 1.5. This is to improve counting statistics during the IR measurement. For lead, the 'natural' isotope abundances are ²⁰⁴Pb (1.37%), ²⁰⁶Pb (25.15%), ²⁰⁷Pb (21.11%) and ²⁰⁸Pb (52.38%), although the natural lead isotope ratios can vary globally from region to region.

The price of enriched lead metal varies depending upon supplier, from 70 ECU/g to as much as 2100 ECU/g. For lead, NIST (Gaithersburg, MD, USA) produce certified enriched lead metal. Samples enriched in ²⁰⁶Pb were purchased from NIST. The calibrants were SRM981 [(²⁰⁶Pb (24.1442%), ²⁰⁸Pb (52.3470%)), SRM982 [(²⁰⁶Pb (40.0890%), ²⁰⁸Pb (40.0954%))] and SRM983 [(²⁰⁶Pb (92.1497%), ²⁰⁸Pb (0.12550%))]. These are referred to as 'natural Pb', 'equal atom Pb' and 'radiogenic Pb' respectively. These were the starting materials for the trialkyllead synthesis and hence the target isotope for enrichment in the IDA process was ²⁰⁶Pb.

The basic method involved the preparation of PbCl_2 from the Pb metal, the preparation of MeMgI or EtMgI (Grignard reagent) and reactions of both to obtain the methyl or ethyl lead compound. The compounds were purified by further recrystallisation from hot ethyl acetate to produce a white crystalline solid. From a 1 g starting material, and after recrystallisation, the final product was only 0.12 - 0.15 g. This indicates a 10-15% efficiency which is disappointing, and is due to the small amount of starting material with associated handling difficulties and the efficiency of the Grignard reaction.

The purity of the calibrants was determined by nuclear magnetic resonance (NMR) spectroscopy (270 MHz, proton, ^{13}C and ^{207}Pb), isotope ratio ICP-MS measurements, HPLC-ICP-MS and flame AAS. For the NMR results the sample spectra were compared to commercially available Me_3PbCl and Et_3PbCl compounds (Alfa Products, Johnson Matthey Ltd, Royston, UK). All spectra were essentially the same and even with 100X scale expansion in the baseline no impurities were found. The isotope ratio measurements by ICP-MS produced no significant differences to the certified ratios in the enriched samples when the certified SRM981 ^{206}Pb to ^{208}Pb ratio was used to standardise the instrument. The HPLC-ICP-MS measurement was qualitative but showed only one peak for each compound and the inorganic Pb content was no different from the blank value. Total lead was determined by flame AAS using the Alfa commercial compounds as calibrants. Seven determinations were separately performed on one of the prepared calibrants to assess statistics for this determination and the result was $100.7 \pm 1.9\%$ (mean \pm standard deviation).

It is worth mentioning that purity studies consume valuable synthesised organometallic standard and in the flame AAS study approximately 14 mg of sample was weighed out and dissolved in deionised/distilled water (10 ml). After dilution this gave approximately $1000 \text{ mg.l}^{-1}\text{Pb}$. The uncertainty in the weighing is significant in this case and of the order of 1-2 %. All the recrystallised compounds synthesised produced purity values between 97-103 %. In all further work the calibrants were considered to be 100 % pure.

In the majority of cases all synthesised compounds were stored in a refrigerator at $2-4^\circ\text{C}$ and in the dark. Prior to using the expensive isotopically enriched lead as the starting material many 'practice runs' were performed to gain experience of the synthesis. A substantial portion (10 g) of the Et_3PbCl compound was left in a cupboard in the dark but at room temperature (25°C). The Et_3PbCl was a pure white crystalline material initially but after 6 months it decomposed and became brown. At first this was observed as a slow colour change from pure white. After 6 months the compound was investigated further. It was found that the brown component (25% in weight) was insoluble in water and is suspected to be lead oxide. An electron diffraction pattern was obtained but this only showed broad band diffuse spectra indicative of a lack of crystalline structure of the decomposition product. The water soluble component showed only Et_3PbCl components by NMR spectroscopy (proton, ^{13}C and ^{207}Pb). A quantity of Et_3PbCl prepared at the same time, but kept in a refrigerator, in the dark, did not show any signs of decomposition. The indications are therefore of a temperature dependent decomposition of Et_3PbCl directly to lead oxide. This tends to support other stability/decomposition studies upon this compound.

16.3.3.2 Preparation of enriched tin isotope standards

Tin has 10 naturally occurring isotopes. These are ^{112}Sn (0.95 %), ^{114}Sn (0.65 %), ^{115}Sn (0.34 %), ^{116}Sn (14.24 %), ^{117}Sn (7.57 %), ^{118}Sn (24.01 %), ^{119}Sn (8.58 %), ^{120}Sn (32.97 %) and ^{122}Sn (4.71 %). The compounds of interest to this study are tributyltin chloride, dibutyltin chloride and monobutyltin chloride, in that order. The price for isotopically enriched tin is greater than that for lead. Statistically, an enrichment isotope with a low (say <10 %) natural isotopic abundance should not be chosen. Given possible isobaric interferences in ICP-MS, choices for tin therefore are limited to ^{116}Sn and ^{118}Sn . On price and availability grounds, ^{116}Sn was selected as the isotope of choice. The proposed synthesis is again via a Grignard reaction.

16.3.4 Data processing in HPLC-ICP-MS and ID-HPLC-ICP-MS

The first aim was to obtain a suitable separation for the target lead species which could then be quantified. With suitable modifications to the HPLC-ICP-MS coupling this was achieved. In this case suitable calibrants of Me_3PbCl and Et_3PbCl were obtained from Alfa Products (Johnson Matthey, Royston, UK). In order to obtain suitable data collection the 'Single Ion Monitoring' software procedure was selected. In this case lead, at m/z 208, was monitored with the dwell time set at 655360μ seconds. The number of channels were varied depending upon the length of the chromatographic run. All other values were as 'default' values. A setting of 1851 channels produced a run time of 1213 seconds. In this mode of operation it is possible to manipulate the chromatogram, and by moving the cursors to the beginning and end of peaks, integrate the peak. Of particular importance is the fact that the peak above the baseline can be integrated and automatic baseline subtraction is possible. The major limitation of this software is that it is restricted to one isotope of one element.

In the case of ID-HPLC-ICP-MS there are some limitations in the software because the software was not designed with this procedure in mind. Software is available for conventional ID-ICP-MS and HPLC-ICP-MS but not ID-HPLC-ICP-MS. In this procedure there are really two different isotope ratio measurements required. Lead is one of those elements that exist in nature with varying isotopic composition because three of the four lead isotopes are daughter nuclides of radioactive decay from U and Th. Consequently, the isotopic composition reflects the relative proportions of U and Th in the parent strata and also the age of the parent strata. Due to this it is really necessary to measure the total ^{206}Pb to ^{208}Pb ratio in the sample by conventional IR-ICP-MS.

The conditions for best precision in IR-ICP-MS has been the subject of study by Sharp and Begely [17]. The optimum conditions for two isotopes were found to be: 10.24 ms dwell time, 3 points per peak, peak jump mode, 1600 sweeps and 10 measurements. The total run time was about 20 minutes per sample. The authors also found that the default quadrupole settle time of 10 ms was not optimum and a value of 2 ms was chosen.

Footnote: For this section in particular readers should be aware that our experience has been with a Fisons Instruments VG Plasma Quad 2 instrument running DOS 3.1 software. Other instruments will have different data processing facilities and different signal measurement criteria.

The second isotope ratio measurement in ID-HPLC-ICP-MS is that of the target species which have been separated. In this case it is necessary to measure both ^{206}Pb and ^{208}Pb . This is possible in the Plasma Quad 2 software due to a program known as 'Time Resolved Data Acquisition (TRA)'. This was designed for multi-isotope/element data collection for transient peaks. As it stands however, there are 3 problems (i) a scan takes up considerable space in the hard disk (ii) in DOS software, the data is stored in machine code (binary) not ASCII format and more importantly (iii) baseline subtraction is not an automatic feature in the software.

In general, there were various possible ways to perform ID-HPLC-ICP-MS given this situation.

- (i) The species can be individually collected directly from the HPLC column and then the isotope ratios can be measured by aspiration of fractions into the ICP-MS.
- (ii) The isotopes can be measured in 2 separate runs using the 'single ion monitoring' mode. In this case automatic baseline subtraction is possible and both peak height and peak area could be obtained.
- (iii) The isotopes can be measured together in one TRA run, however, it is necessary to manually subtract the baseline or run separate blanks. Peak height and peak area could be obtained.
- (iv) The isotopes can be measured together in one TRA run which can then be stored on a floppy disk. The TRA binary file can be converted into an ASCII file which can then be read into a data processing package for integration with an automatic baseline subtraction routine.
- (v) Write a new software program incorporating all of (iv) plus the ID calculation procedure.

The approach adopted in this project was that of a coupled on-line system for ID-HPLC-ICP-MS therefore method (i) was not considered to offer any advantage. Method (ii) defeats some of advantages of the ID approach in that the real aim is a simultaneous measurement of two isotopes in the same sample run. Method (iii) has practical problems with manual baseline subtraction or separate blank runs. For example, what would be a representative baseline? How wide was the peak? Further, problems arise with gradient elution chromatography and sloping backgrounds as discussed earlier.

The approach currently adopted is that of (iv) with (v) being investigated. In order to enable method (iv) to succeed it was necessary to write a software program to convert the TRA files to ASCII. In the new OS2 software from Fisons Instruments the TRA data are stored as ASCII. A software programme called Fig P (Biosoft, Cambridge, UK) was utilised to draw the TRA data as a chromatogram and to integrate the peaks with baseline subtraction. The ratios were then calculated and manually incorporated into the ID-HPLC-ICP-MS method. The Fig P program is relatively inexpensive and is really designed as a scientific graphics package with the AUC (area under curve) feature added as an added extra. The current method does work but is time consuming and attention is now focused upon developing method (v).

The approach adopted for the data collection in TRA is based on relatively fast peak jumps from ^{206}Pb to ^{208}Pb with dwell times of $1280\ \mu\text{s}$ and many peak jump sweeps (50) to obtain a datum point per second. At present the quadrupole settle time is set at 1 ms.

16.3.5 The determination of trimethyllead by HPLC-ICP-MS in a rainwater sample

A rainwater sample was received for analysis for trimethyllead determination as part of an intercomparison exercise organised by the BCR of the EC. The sample was analysed by 15 laboratories throughout Europe using different analytical techniques. As supplied, the sample required dilution of 1000 fold to give an approximate value, in region of $50\ \mu\text{g.l}^{-1}\text{Me}_3\text{PbCl}$ present in an excess of inorganic Pb.

Since the levels of Me_3PbCl were low, problems were expected with the drifting baseline using the gradient elution program. This was investigated using a solution containing only $50\ \mu\text{g.l}^{-1}\text{Me}_3\text{PbCl}$. The sample volume was $200\ \mu\text{l}$. The first program investigated included a 4 minute isocratic stage using a 10:90 methanol to buffer ratio to elute the inorganic Pb, and then a gradient from 4 to 7 minutes to a 30:70 ratio to elute the organolead compound. The result from this is shown in Figure 6A. The Me_3Pb^+ peak is point 3 on the chromatogram. Points 1 and 2 were reproducible between different chromatograms when samples were loaded onto the HPLC column. The baseline fluctuation at point 1 corresponds to the sample injection time and was due to a 'system peak'. The small pressure pulse due to the sample switching valve possibly changes the plasma stability for a period producing this effect on the baseline. At point 2 in Figure 6A the gradient is beginning to effect the methanol content in the plasma and hence effect the baseline as discussed earlier. With respect to the separation and determination of Me_3Pb^+ from Pb^{2+} these baseline fluctuations are irrelevant. What is relevant about this chromatogram is the fact that the baseline underneath the Me_3Pb^+ peak is not linear. This could produce errors in integration. The situation was worse with a 'step gradient' to a 30:70 ratio, with the Me_3Pb^+ eluting further up the sloping baseline. It became apparent that the gradient elution program must be constructed in such a way that when the Me_3Pb^+ peak eluted, the baseline must be at least linear for quantitative purposes. This required further compromises between peak breadth and elution time in order to obtain a suitable linear baseline.

Figures 6B and 6C show the results of such a compromise gradient program. In this case the program began with a 10:90 methanol to buffer ratio for 2 minutes; a step to 20:80 for 2 minutes; a step to 25:80 for 1 minute and then a slow gradient over 5 minutes to a 30:70 ratio and held for 5 minutes. Figure 6B shows the blank chromatogram with the 'system peak' gradient curve and, towards the elution time of the Me_3Pb^+ peak (see Figure 6C), a long linear baseline. The chromatogram in Figure 6C was obtained from the diluted rainwater sample sent for analysis.

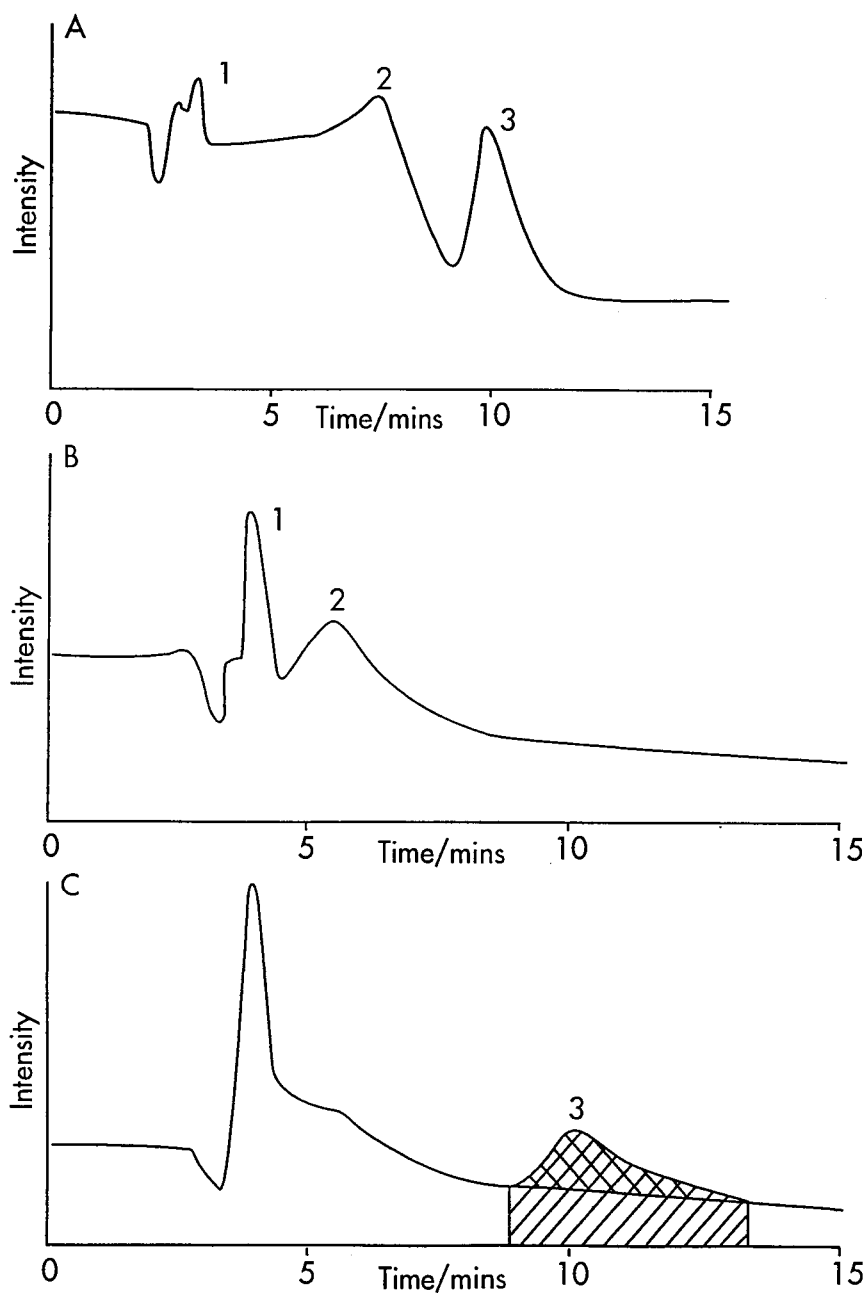


Figure 6: HPLC-ICP-MS chromatograms using gradient elution. Points 1, 2 and 3 show 'system peak', methanol gradient and Me_3Pb^+ peak respectively. Chromatogram A highlights the need for a linear baseline elution of the Me_3Pb^+ species. B- sample blank under optimised gradient and C- rainwater sample analysed to assess accuracy of proposed procedure.

The analysis was carried out using standard ICP-MS operating conditions (forward power 1.5 kW, reflected power <5 kW, argon outer gas flow of 15 l min⁻¹, auxiliary gas flow of 0.7 l min⁻¹, and a nebuliser gas flow of 0.73 l min⁻¹). The 'single ion monitoring' software routine was used with a dwell time of 655,360 μ seconds at m/z 208. A blank and 3 standards containing 0, 28.1, 55.5 and 76.7 ng.g⁻¹ of Pb, as Me₃PbCl, were run in duplicate for calibration. Five separate aliquots of the sample were analyzed in duplicate, interspersed between either a blank or calibrant (55.5 ng.g⁻¹). The results obtained are shown below. The mean result obtained for the five separate sample aliquots was 45.0 \pm 1.94 ng.g⁻¹ which compares favourably with the mean of values found by other laboratories of 42.7 \pm 4.8 ng.g⁻¹ [18]. The blank produced a total integrated background count of 5596 with a standard deviation of 1867 counts (n = 6). The results for standard 2 (55.5 ng.g⁻¹ Pb as Me₃Pb⁺) gave a mean signal of 346,645 counts with a percentage relative standard deviation of 0.68 % (n = 6). The detection limit, based on 3 times the standard deviation, was 0.48 ng.g⁻¹ Pb (total) as Me₃Pb⁺.

Results for the Rainwater Sample for Me₃Pb⁺

Sample No	Mean Net Signal/ Counts	Element Concentration/ ng g ⁻¹	RSD% (n = 6)
1	285,173	46.5	6.2
2	292,008	47.0	4.7
3	303,035	45.6	2.0
4	274,914	43.0	2.8
5	259,676	42.9	4.9

These results indicate that the HPLC-ICP-MS determination of Me₃Pb⁺ is possible and that adequate accuracy can be achieved with commercially available standards. The next step was to incorporate the complete ID-HPLC-ICP-MS procedure and this is discussed in the next section.

16.4 Evaluation of the ID-HPLC-ICP-MS technique

The complete ID-HPLC-ICP-MS procedure has been developed using standards of Me₃PbCl synthesised from the NIST SRMs 981, 982 and 983 as discussed previously. Three solutions were prepared that contained approximately 50 mg.l⁻¹ Pb as Me₃PbCl. To minimise potential baseline problems the chromatography was isocratic, 40:60 methanol to buffer mixture. The ICP-MS instrument was optimised for Pb in the same mixture.

The test solutions were prepared by adding a small quantity (typically 30-50 μ l) of the SRM981 Me₃PbCl solution to 10 ml buffer. This represented a sample with 'natural' Pb isotopic abundance. The 'spike' was 20-40 μ l of either SRM982 or SRM983 synthetic Me₃PbCl added to the sample solution. Under these controlled criteria, accuracy and precision data were acquired.

The data acquisition and processing used has been discussed earlier. On the ICP-MS instrument used, the TRA software was utilised. Lead, at m/z 206 and 208, was monitored. The software was set for 1280 μs dwell time at each isotope with 50 peak jump sweeps (the quadrupole settle time was 1 ms). This was chosen for two reasons. Firstly, the relatively fast modulation between each isotope, although limited by the quadrupole settle time, should reduce noise in the measurement. Secondly, these conditions produced a real measurement time of approximately 1 second and this seemed an acceptable time period to define an HPLC peak. From the same logic, GC peaks may require different acquisition times since they are, in general, faster peaks. In this case the number of sweeps should possibly be reduced rather than the dwell time since it is the quadrupole settle time that is the limiting time. Further work will be required to establish experimentally how critical these parameters are to isotope ratio (IR) precision measurements in coupled chromatography ICP-MS.

Once the TRA data had been acquired it was stored to hard disk and then converted to ASCII files using an in-house written program. Once in ASCII format, the files were read into the Fig P software package (see section 16.3). Figure 7 shows a TRA scan using the Fig P graphics package. The ^{208}Pb peak has been artificially translated in the 'Y' axis for visual clarification only. The Fig P software package allows a cursor to be moved under the peak and to define the start point and end point of the peak to be manually selected for integration. In the top right hand side of the video display screen the exact points selected are shown. Therefore, it is possible to select the exact same points of start and end between the two isotope chromatographic peaks and eliminate uncertainty. The isotope ratios are simply calculated from the baseline subtracted integrations of the two isotopic peaks.

The principle of isotope dilution analysis (IDA) has been discussed earlier in this book by Campbell *et al.* The equation shown for C_x , the concentration of analyte in a sample, is one of the many forms available and each paper on this subject seems to present a different equation. The equation used by Fassett and Paulsey (22) was used in this study and is shown below. This equation was chosen because it is in a simple form which can be readily incorporated into a computer program or 'spread sheet'.

$$C_x = \frac{(C_s W_s) (A_s - R_m B_s)}{(W_x) (R_m B_x - A_x)}$$

where C_x = concentration of analyte in the sample

W_s = weight of spike

W_x = weight of sample

C_s = concentration of spike

A_s = atom fraction of isotope A in the spike

B_s = atom fraction of isotope B in the spike

A_x = atom fraction of isotope A in the sample

B_x = atom fraction of isotope B in the sample

R_m = measured isotope ratio in the spiked sample

Traditionally, the units have been defined in mass terms *i.e.* $\mu g \cdot g^{-1}$ or g however, if all solutions are aqueous then it can be assumed 1 ml = 1 g and this eliminates the need for weighing (especially the small weight, W_s) and accurate micro pipettes can be used. In this study a further assumption was made in that the atom fractions of isotopes A and B *i.e.* ^{206}Pb and ^{208}Pb were those of the certified NIST lead standards.

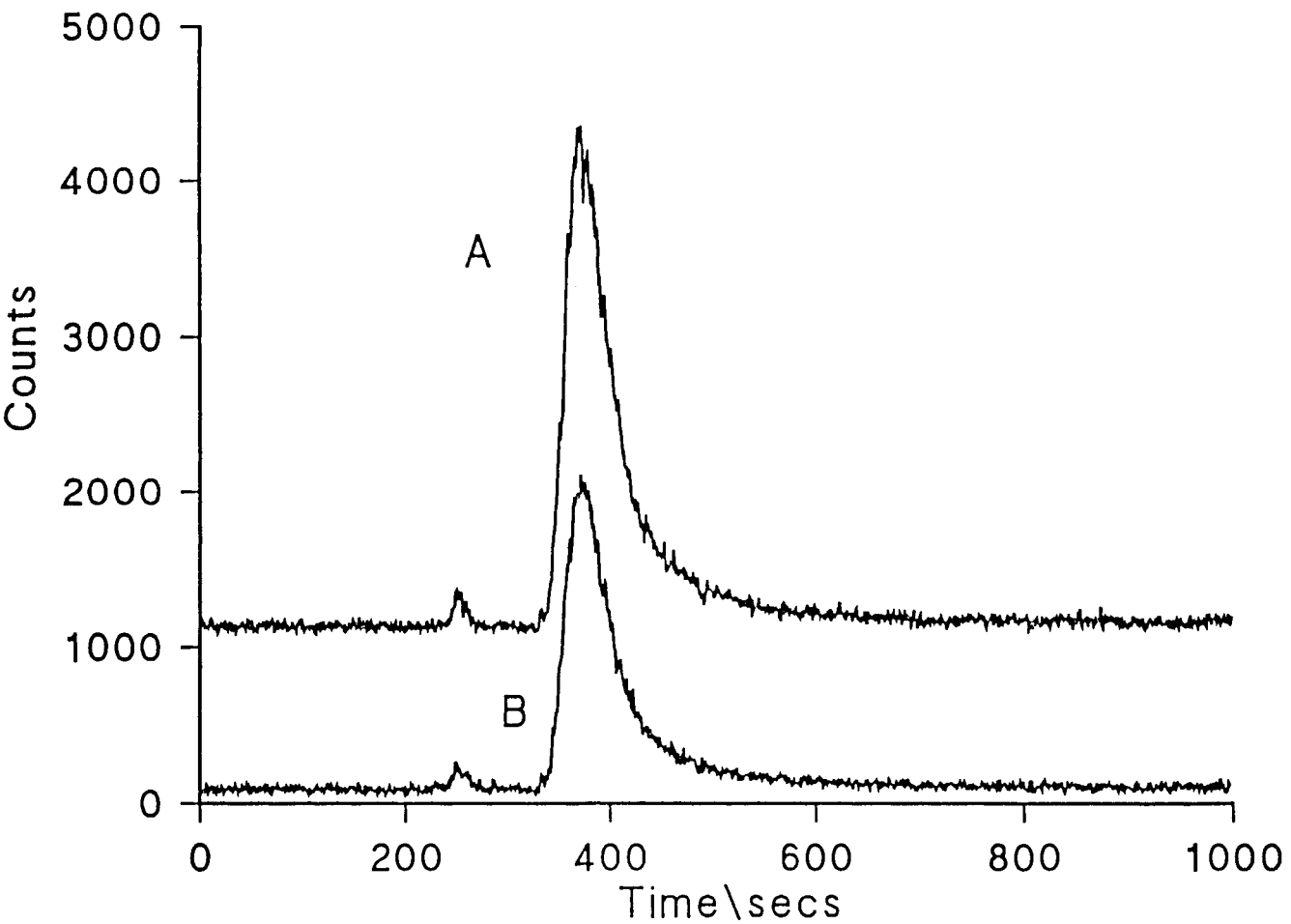


Figure 7:

HPLC-ICP-MS chromatogram of A- ^{208}Pb and B- ^{208}Pb as Me_3Pb^+ after incorporating the TRA scan into the Fig P graphics software. The ^{208}Pb peak is shifted vertically for visual reasons only.

16.4.1 Results

The aim of the initial evaluation was to establish precision and accuracy data and to investigate any trends found, especially precision in IR measurements. A series of five measurements are shown below for one spiked sample which clearly shows a sensitivity drift over the one hour taken to obtain this data. The total Me_3PbCl concentration was about $250 \mu\text{g.l}^{-1}$ and the sample volume was $200 \mu\text{l}$.

	^{206}Pb	^{208}Pb	$\frac{^{206}\text{Pb}}{^{208}\text{Pb}}$
Run 1	123261	202854	0.608
Run 2	126167	201411	0.626
Run 3	135173	223029	0.606
Run 4	135353	223239	0.606
Run 5	138406	224296	0.617
Mean	131672	214966	0.613
RSD %	4.46	4.98	1.28

The main conclusion from this set of data is that the individual precisions of ^{206}Pb and ^{208}Pb are about 5 % whereas the isotope ratio precisions are closer to 1 %. These results indicate that IR measurements are more precise than individual isotope measurements and this is in agreement with conventional IR measurements in ICP-MS. A further practical conclusion is that the data collection and processing ideas developed work in an experimental set-up.

A further set of data with 8 separate measurements ($n=8$) produced a precision of 5.73% for ^{206}Pb and 5.40 % for ^{208}Pb with an isotope ratio precision of 1.80 %. Again, these results show the same trend. In general, it was expected to be the case that precision in HPLC-ICP-MS would be governed by the usual precision of manual injection onto an HPLC column. These are typically quoted as 2-5 %. It is expected that isotope ratio measurements in HPLC-ICP-MS will improve this precision, not only within one sample run but also between sample runs, since the ratio is independent of sample size.

To assess accuracy, two artificial samples were spiked with the Me_3PbCl synthesised from SRM 983. The first sample produced an isotope ratio of 1.566 which corresponded to a concentration C_x of 0.270 mg.l^{-1} . The expected value was 0.285 mg.l^{-1} . The second sample produced an isotope ratio of 2.058 which corresponded to a concentration C_x of 0.248 mg.l^{-1} . The expected value was 0.228 mg.l^{-1} . The measurements were made in duplicate. These values are acceptable considering the early stage of the method evaluation. Also, certain minor errors, such as mass bias have not yet been taken into account.

Further work is continuing to assess the factors that affect accuracy and precision and the final method developed will be used in intercomparison exercises and certification work within the new Measurement and Testing Programme of the European Commission [18].

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17.

Speciation of organotin compounds in environmental samples by GC-MS

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The total worldwide consumption of organotin compounds has dramatically increased in the last thirty years from about 5,000 tons per year at the beginning of the '60s to over 60,000 tons per year in the midst of '80s. They are mainly used as stabilizers for rigid PVC (mono- and di-organotins) and as biocides (triorganotins). Even if the use as biocides accounts for only 30 % of the total world consumption, it contributes, due to the direct introduction, to the largest portion of organotins in the environment. Furthermore, the total production of organotins in the last thirty years increased by about 10-fold while the production of triorganotins for biocide uses has increased by about 20-fold in the same period. The environmental aspects of non-biocidal organotin compounds has been recently reviewed by Maguire [1]. In the conclusions it is stated that the most important non-pesticidal route of entry of mono- and dimethyltin, butyltin and octyltin to the environment is through leaching of PVC by water. Triorganotin biocides are used in pesticide formulations (mainly triphenyltin (TPhT)) [2] and, above all, in antifouling paints (mainly tributyltin (TBT) but also, increasingly, TPhT) [3]. Triphenyltin acetate (Brestan™) and triphenyltin hydroxide (Duter™) are used for the control of *Phytophthora* infestants, tricyclohexyltin hydroxide (Plictran™) for the control of *Phytophagous*; 1-tricyclohexylstannyl-1,2,4-triazole (Peropal™) and triphenylbutatin oxide (Vendex™) are both used as miticides [5]. These products are largely used in agricultural application and contamination could result from run-off water and overspray. Tributyltin-based antifouling paints were introduced at the beginning of the 60's but their widespread use started only in the 70's, replacing copper-based paints due to a superior performance: TBT paints are effective for about 5-7 years while copper paints are effective for no more than two years [4-5].

There are two types of organotin based antifouling paints: (i) conventional or "free association" paints in which the toxicant is loose in the paint and (ii) non conventional or polymeric paints in which the toxicant is chemically bound to a polymeric matrix. Conventional paints are more polluting and have a lower effect duration than polymeric paints, having a higher TBT release rate [6].

TBT is directly released into aquatic environment and its immission can be both continuous (release from the hulls of the boats) or intermittent (release from dockyard activities as paint removal, cleaning, painting, *etc.*). Environmental persistence and fate of TBT are strictly correlated to the specific characteristics of the aquatic ecosystem such as temperature, salinity, pH, suspended matter, microbial populations, flushing rates, *etc.* Distribution of TBT among the different environmental compartments is regulated by (i) physical mechanisms (including volatilization, adsorption, *etc.*), (ii) chemical mechanisms (including photochemical reactions) and (iii) biological mechanisms (including uptake and transformation) [7].

Both TBT and TPhT undergo degradation processes in marine environment, such as microbial and UV degradation, consisting in a progressive dealkylation down to inorganic tin [8]. Sufficient evidence exists of a faster rate for the DBT \rightarrow MBT degradation in some experimental and environmental conditions [9-11]. As the toxicity of the organotins is maximum for the trisubstituted compounds, the degradation can be considered as a mechanism of detoxification. In fact, elemental Sn and its inorganic compounds are practically non toxic for all living systems: due to their very low solubility in lipids, they are scarcely accumulated by the organisms [12-13]; furthermore, at physiological pH, the element is not reactive and its oxides are practically insoluble [14].

On the contrary, the progressive introduction of organic groups at the Sn atom exerts a profound influence on chemical-physical properties, biological activity, mobility and persistence. This leads to an increasing toxicity of the molecule, reaching a maximum for the trisubstituted compounds [15-16]. For marine organisms the highest toxicity is shown by tributyl, triphenyl and tricyclohexyltin compounds [17]. The inorganic substituents do not significantly affect the toxicity of the compounds, unless they are strongly coordinating groups [18-20]. The relative lipophilicity of triorganotin compounds as long as the tendency to bind with complex and simple lipids, make them able to cross biological membranes, producing toxic effects. Many reviews containing toxicological data on organotin compounds have been published [19-23].

The bioaccumulation process depends on the lipophilicity of the substance and on its resistance to metabolism and excretion processes [24]. Studies of kinetics and mechanism of accumulation showed that marine bivalves rapidly and effectively accumulate organotins even when exposed to low concentrations of dissolved material [25]. Bivalves accumulate dissolved TBT from sea water, presumably directly into exposed tissues such as gills, followed by migration to other tissues, or by ingesting tainted food. Very high concentrations can be reached in these organisms, because they are not capable, due to a low activity of the mixed function oxidase system, to metabolize a wide range of xenobiotics, including organotins [26-27].

Laboratory experiments on the accumulation of TBT demonstrated high bioconcentration factors for oysters [28] and for mussels [29-30]. Bioconcentration factors calculated in the field [11, 23, 31-32] resulted to be even higher than those predicted on the basis of octanol-water partition coefficient or calculated from laboratory experiments. The reasons for the discrepancies between laboratory experiments and field data are probably to be found in the difficulty of considering all the important parameters regulating the environmental behaviour in laboratory experiments.

The direct introduction in the marine environment and the successive accumulation together with the high toxicity of these compounds towards "non-target" organisms, such as oysters and mussels, can cause environmental and economic damages as observed in the past in the Arcachon Bay (France) [33].

France was the first country to restrict the use of the TBT based antifouling paint by a legislation that regulated the use of these paints on boats with hulls less than 25 m long [34]. Similar regulations were enacted by many other countries such as UK, USA, Canada, *etc.*

Recently, recommendations to extend the restriction to all organotin paints not only for boats, but also for industrial water cooling systems, mariculture structures, *etc.* were taken into account. Many references on organotin legislative information can be found in literature [6, 34-37].

17.1 Analytical methods for the environmental analysis of organotins

Many analytical methods have been developed to determine organotin compounds in environmental matrices and several monitoring programmes have been carried out in order to evaluate the effectiveness of legal provisions. As the environmental quality target (EQT) for TBT in water is generally put at the ng.l^{-1} level, analytical methods should be sensitive enough to detect organotin compounds down to these very low concentrations. Furthermore, these methods should be able to discriminate among different chemical forms providing information on the speciation of organotin compounds. In fact, due to the wide differences in toxicity among tin species, speciation is an invaluable tool to understand the distribution and fate of these compounds in the environment and to assess the environmental risks in the studied areas.

Obviously, analytical methods should be characterized by good precision and accuracy, avoiding mistakes that could lead to wrong environmental considerations.

It is worth to point out that mistakes occur in the whole analytical procedure and not only during the instrumental measurement steps. Sampling, storage of samples, sample pre-treatment and analytical measures are all critical stages in speciation studies.

Various papers discuss errors occurring during sampling [38-39] and storage [10, 40].

Sample pre-treatment generally involves an extraction (in order to separate the analytes from the matrix and concentrate) and a derivatization/clean-up step (in order to improve analytes detection).

17.1.1 Extraction

A large number of extractants have been used for the extraction of organotins from water, sediment and biological samples: acids [41-42], organic solvents with [43-46] and without [47-49] complexing agents (*e.g.* tropolone) and a mixture of them [50-56].

Recently, a supercritical fluid extraction was developed for the determination of TBT in sediments [57]. This development is described in detail in Chapter 18 of this book.

Low extraction yields, losses of analytes and contamination directly affect the quality of the results. Moreover, change in the organotin speciation occurring during this step lead not only to wrong information about the contamination levels but also to wrong considerations about the extension of the ongoing degradation phenomena. Thus, recovery should be carefully tested for each organotin compound. In absence of reference materials, spiking experiments are generally used. However, the adequacy of such experiments to represent a realistic surrogate is still a matter of controversy [39] and further studies are needed.

17.1.2 Derivatization

As said in previous Chapters, the most used derivatization methods in organotin analysis are hydride generation and alkylation with Grignard reagents.

Hydride generation [41,49,55,58-62] is generally used when the final determination is performed by atomic absorption spectrophotometry (AAS) while using flame photometry detector (FPD) or mass spectrometry detector (MS) Grignard derivatization [31, 44-46, 63-68] is generally preferred.

Hydride generation seems very attractive for butyltins analyses, even if poor recoveries are obtained from sediments containing high sulphur, hydrocarbons and chlorophyll content due to an inhibition of the hydride generation [69,70]. Determination of phenyltin species is generally hindered due to the low yields and poor reproducibility of hydridization. Furthermore, losses of the highly volatile mono-alkyltin species could occur if the analytical method requires a concentration step by evaporation of the final solution. Problems concerning the hydride generation for the determination of butyltin compounds are treated in detail in Chapter 19 in this book [71].

Grignard derivatization is generally performed by methylation [63,64], ethylation [44], pentylation [55,66,68], hexylation [31,67], *etc.*. Pentylation seems to offer the best compromise to obtain non-volatile derivatives and good gaschromatographic properties both in terms of separation and detectability (see also section 17.1) [66]. Grignard derivatization needs more steps than hydride generation *i.e.* the reagent destruction (usually performed by careful addition of water and then of a strong acid) and the back-extraction of the alkylated organotins, increasing risks of contamination, decomposition, losses, *etc.*

Recently, a new derivatization method with sodium tetraethylborate was proposed for butyltin compounds [70]: its characteristics are situated somewhere in between those of hydridization and Grignard alkylation.

Derivatization yields should be carefully evaluated but the absence of commercially available derivatized compounds of proven purity makes it a difficult task. The use of radiolabelled compounds and/or synthesized derivatized standards in one's own laboratory [72] should be considered.

17.1.3 Analytical techniques

Chromatography is the most cited analytical technique for the determination of organotins. Atomic absorption spectrometry (AAS), flame photometry (FPD) and mass spectrometry (MS) are mainly used as detection system. Chromatography interfaced with atomic absorption spectrophotometry has been reviewed by Donard and Pinel [73]. Several papers describing analytical procedures based on FPD determination are reported in literature [64, 68, 74-78]. Mass spectrometry detection is described in detail in the next paragraph including literature references. Electron capture (ECD) [79-81] and flame ionization (FID) [82] detectors have been also used. The application of gas chromatography atomic emission spectrophotometry (GC-AED) [83] and of high performance liquid chromatography interfaced with isotope dilution - inductively coupled plasma mass spectrometry (HPLC/ID-ICP/MS) for tin speciation is described in Chapter 16 of this book [84].

Nevertheless, a number of other techniques, such as graphite furnace atomic absorption spectrophotometry (GFAAS) [85-89], voltammetry [90-93], fluorimetry [94-95] and spectrophotometry [96-97] are present in literature.

17.2 GC/MS analysis of organotins

Mass spectrometric detection systems have distinct advantages over the other specific detectors that have been used for organometallic compounds analysis (like AAS, ICP, AED, etc.):

- 1) they provide both sensitivity and selectivity together with structural confirmation capabilities;
- 2) coupling to HRGC is straightforward and there is no need to "adapt" each other different instruments;
- 3) modern instrumentation is robust, easy to use, relatively inexpensive (benchtop instruments) and is now really at an industry-standard level of reliability;
- 4) there is no need to be a "MS specialist" to perform analyses.

Mass spectrometry is particularly well suited for the analysis of organometallic species (provided they are amenable to GC): the metal atom (particularly the heaviest ones) gives the compound an "extra-mass" with respect to the possible coeluting organic compounds, making detection and confirmation easier and less prone to interferences. This obvious advantage is not confined to GC/MS only, but is also the key feature of such powerful detection techniques as He-MIP/MS [98], ICP/MS [99-102], ionspray MS/MS [50], laser ionization/TOF-MS [103], etc.

17.2.1 Which MS detector ?

The correct choice of a MS detector is usually a complex task, involving both technical and budget aspects. Generally speaking, one should answer some questions:

- Do I need a mass spectrometer or a mass spectrometric detector ?
- Do I really need high mass resolution ?
- Do I need extra features (NICI, PICI, LC interfaces, solid probes, FAB, *etc.*) ?
- Is not productivity one of my major goals ?
- Do I have well trained personnel and strong technical assistance support ?

If the answers were mainly no, a benchtop quadrupole MS detector is the best choice, progressively upgrading to high-costs, complex and technically demanding magnetic sector HRMS spectrometers.

In the field of organotin compounds analysis the MS system of choice is by far the quadrupole type: high mass resolution is not needed and a "workhorse" as the quadrupole is important when one is frequently facing relatively "dirty" samples as those from environmental matrices. The ion trap detector is also gaining relevance as an all-purpose mass detector, after some technical drawbacks that affected the oldest versions of the instrument were partially solved.

17.2.2 Gas chromatographic characteristic of organotins

The tremendous separating power of capillary gas chromatography is fully exploited if the analytes are in a suitable chemical form in order to match the technical characteristics of the injection system, the capillary column and the detection system. Various authors reported results of investigations on the chromatographic behaviour of organotin derivatives [76, 104-106] and even if the analysis of organotin chlorides and hydrides were found feasible, the range of applicability of Grignard derivatization is by far superior for a number of reasons: it gives extremely stable compounds and the derivatized samples can be easily stored for later analysis or other investigations;

- Grignard derivatives can be obtained also from extracts of extremely dirty samples;
- Grignard derivatives can be obtained from almost the whole range of organotin compounds, whereas useful chlorides and hydrides are limited;
- Grignard derivatives show excellent chromatographic properties on the most common capillary phases;
- samples after derivatization are easily subjected to cleanup to improve chromatography and detection.

The Grignard reagent of choice is, in our opinion, *n*-pentylmagnesium bromide for various practical reasons:

- it is less reactive than *e.g.* methylmagnesium bromide, but is less hazardous and still sufficiently strong to act on dirty extracts;

- it gives derivatives of optimal volatility to be analyzed on "all-purpose" columns; the derivatives show a wide chromatographic separation;
- it allows a good separation of butyltins from phenyltins to be achieved.

The fully derivatized organotins behave chromatographically like hydrocarbons and their separation is perfectly achieved on nonpolar phases of the SE-30 (methylsilicone) or SE-54 (methyl-5 % phenylsilicone) type. Capillary columns with phase ratios from 200 to 450 can be successfully used. Since separation of the compounds is easily achieved, column length and thermal program are not critical: relatively short columns (15-25m) and fast thermal programs (8-10°C/min increase) can significantly speed analysis and improve sensitivity. An example of a typical pentylated organotins chromatogram is shown in Figure 1.

The good thermal stability and absence of active groups in the Grignard derivatized compounds allow the use of rugged inlet systems like the split-splitless one, whereas chlorides or hydrides are amenable to cool on-column injection in deactivated precolumns only. The split-splitless injector is much more resistant to the buildup of involatiles and it is particularly suited for environmental samples, even if it has a lower reproducibility than the on-column one.

The use of an internal standard is always advisable in quantitative GC and is simply mandatory for GC/MS. Due to the complex manipulation of the samples, recovery standards (surrogate compounds) analysis is highly desirable. The most commonly used compound is TPrT, which is usually added to the sample at the beginning of the whole procedure and therefore acting as a surrogate compound. Because of the very good recoveries of trisubstituted compounds, TPrT is also considered as the internal standard for the quantitative calculations. A more rigorous approach would be to add a complete set of surrogate compounds (for mono- and disubstituted species too) to the sample and to add a true internal standard (e.g. tetrabutyltin) to the final solution. To our knowledge, this approach has never been fully addressed by the scientific community, probably because of the lack of pure reference compounds.

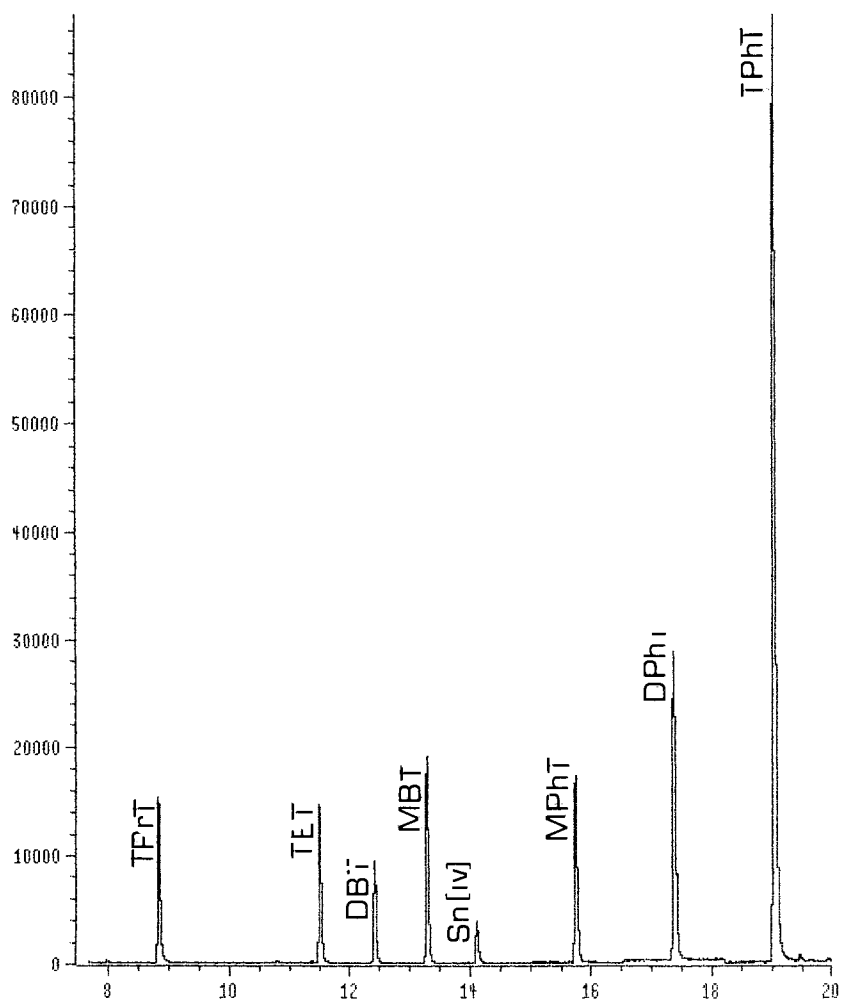


Figure 1: GC-MS of pentylated organotin compounds standard mixture. Amounts injected on column: TPrT, 0.20 ng; TBT, 0.17 ng; DBT, 0.12 ng; MBT, 0.20 ng; MPhT, 0.12 ng; DPhT, 0.11 ng; TPhT, 0.12 ng.

17.2.3 Mass spectral characteristics of organotins

Various papers report mass spectral data for organotin compounds [63,72,76,104,107-110], but it is always worth to remind their key MS characteristics.

Tin has a "rich" isotopic composition as shown below:

Mass number	Abundance (%)
116	14.30
117	7.61
118	24.03
119	8.58
120	32.85
122	4.72
124	5.94

Natural abundances of main isotopes of Sn

This isotopic distribution gives a peculiar aspect to the MS spectra of tin compounds: each fragment ion appears as a cluster of m/z values with the above mentioned ratios. From the analytical point of view, this improves identification of the analyte but decreases GC/MS sensitivity because the ions produced by a single fragmentation are spread over a range of m/z .

The fragmentation pattern under classical EI conditions of fully alkylated organotins (which are the most frequently analyzed derivatives: hydrides are rarely used [105] unless for methyltins [111]) is quite simple, consisting in a stepwise loss of the alkyl (aryl) groups. Examples for the pentylated organotin compounds considered in this chapter are shown in Figures 2-8. The molecular ion is virtually absent. Losses of alkyl groups seem to be almost equiprobable (at least in the C3-C5 range) and the structure of the compound can be deduced by mass losses and by ratios of the relative isotope clusters. The presence of aryl groups gives additional stability to fragment ions. The case of TPhT is particularly significant,

where the cluster originating from the loss of the pentyl group is the only present, despite the 3:1 statistical favour to the loss of a phenyl. Different ionization modes as PICI [112] give a predominant molecular cluster ion, useful for identification and confirmation purposes, but when coupled to GC, this approach gave higher detection limits than the usual EI-SIM.

The scan mode detection of quadrupolar detectors cannot cope with the low environmental concentrations of organotins (the ion trap detector could be a valuable alternative for high sensitivity scan) and diagnostic ions have to be chosen for SIM.

As a general rule, diagnostic ions for SIM have to fulfill some basic requirements: they should be among the most abundant ions of the spectrum in order to increase sensitivity;

- they should be unique and characteristic of the compound in the matrix under analysis;
- the selected m/z should have the lowest possible instrumental noise in the experimental setup;
- m/z values whose ratios are checked for confirmation should be isotopic peaks rather than different fragment ions.

In the case of pentylated organotins these requirements are easily met thanks to the simple fragmentation and the peculiar isotopic composition.

The GC separation of pentylated organotins is very good, as shown before, and the number of ions to be monitored in each time window can therefore be kept to a minimum, again increasing sensitivity.

In our experience, organotin analyses by GC/MS rarely suffer from serious interferences: the only problem could be an overload of the ion source from high amounts of coeluting compounds in case of extremely polluted samples (petroleum hydrocarbons in harbour sediments or lipids from non perfectly cleaned-up biological extracts). In those cases a retention times shift is usually evident, due to the overloading of the capillary column.

GC/MS data are ideal for the implementation of automatic data reduction and interpretation routines: integration of ion chromatograms, peaks detection and identification, identity confirmation and quantitation can be performed in an automatic way, using the programming capabilities of modern GC/MS workstations. Care should be taken in revising the results so generated, as the integration of different ion traces is the weak link in the automation: widely different noise or interference level are not uncommon even for $Dm/z=2$, which is the case of organotins, seriously affecting data quality. Current integration software is not sufficiently intelligent to cope with these cases and nothing can still replace the expert's eye evaluation capabilities.

The other great advantage of the MS detector (and particularly the quadrupolar one) is its wide linear range (up to 7 decades) which makes calibration with the internal standard method an easy task: the injection of two standard solutions (bracketing the expected amounts to be analyzed) is sufficient to verify the constancy of the response factors. The repetition of the standards injection between samples ensures that the whole system is working properly and results are trustable.

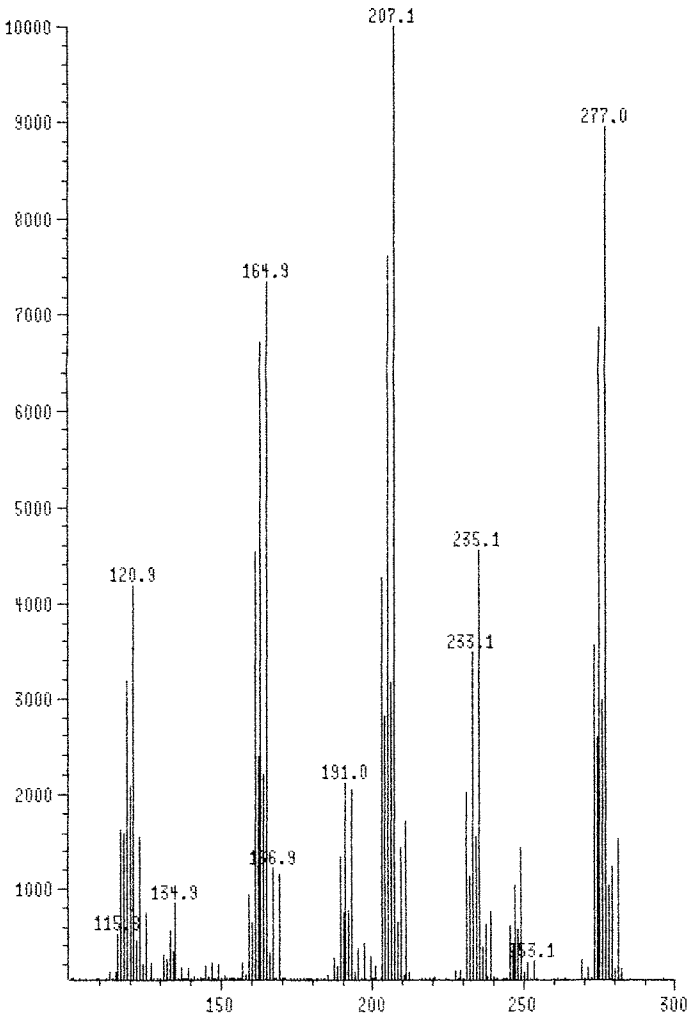


Figure 2: Mass spectrum of TPtT

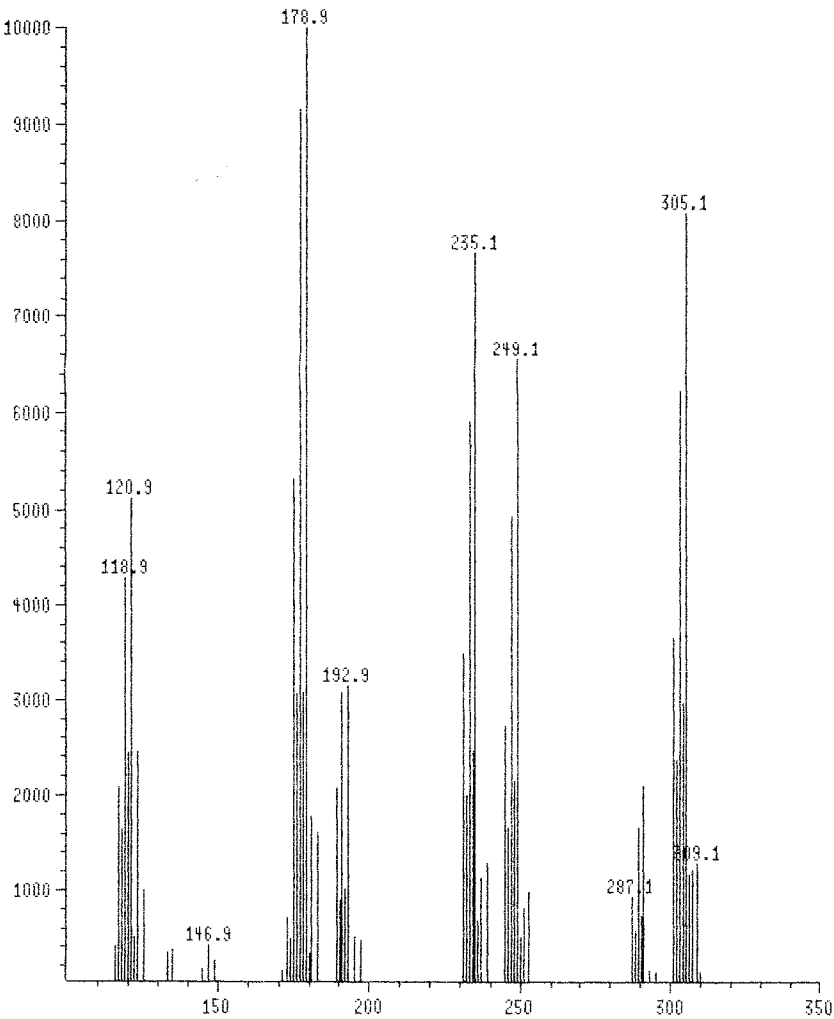


Figure 3: Mass spectrum of TBT

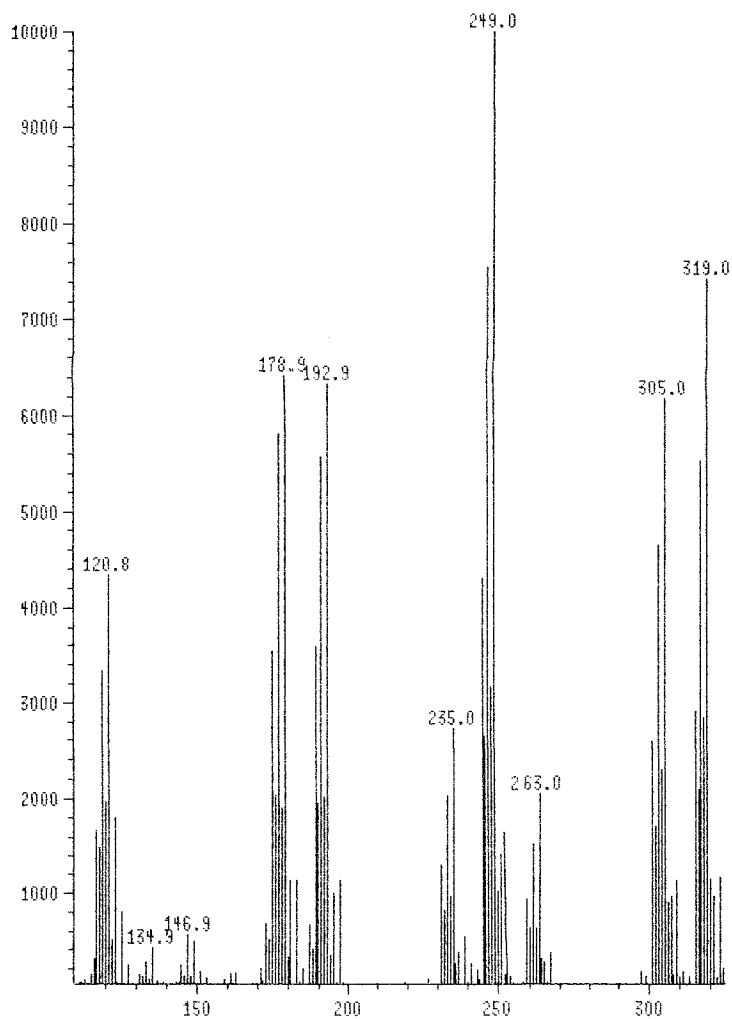


Figure 4: Mass spectrum of DBT

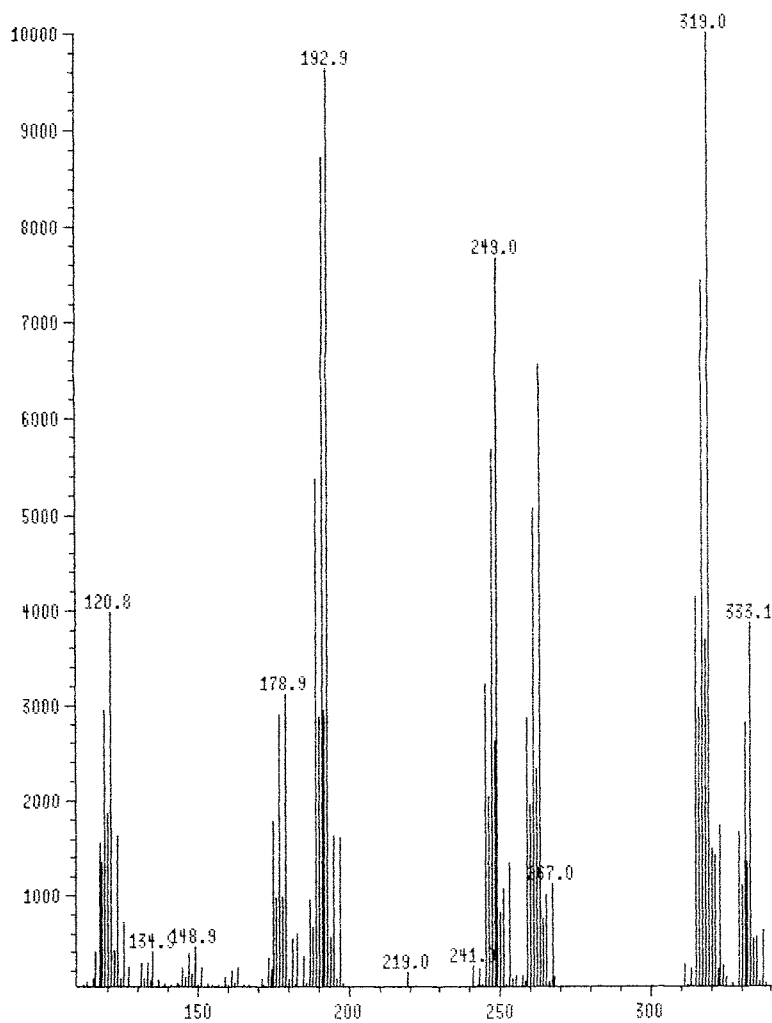


Figure 5: Mass spectrum of MBT

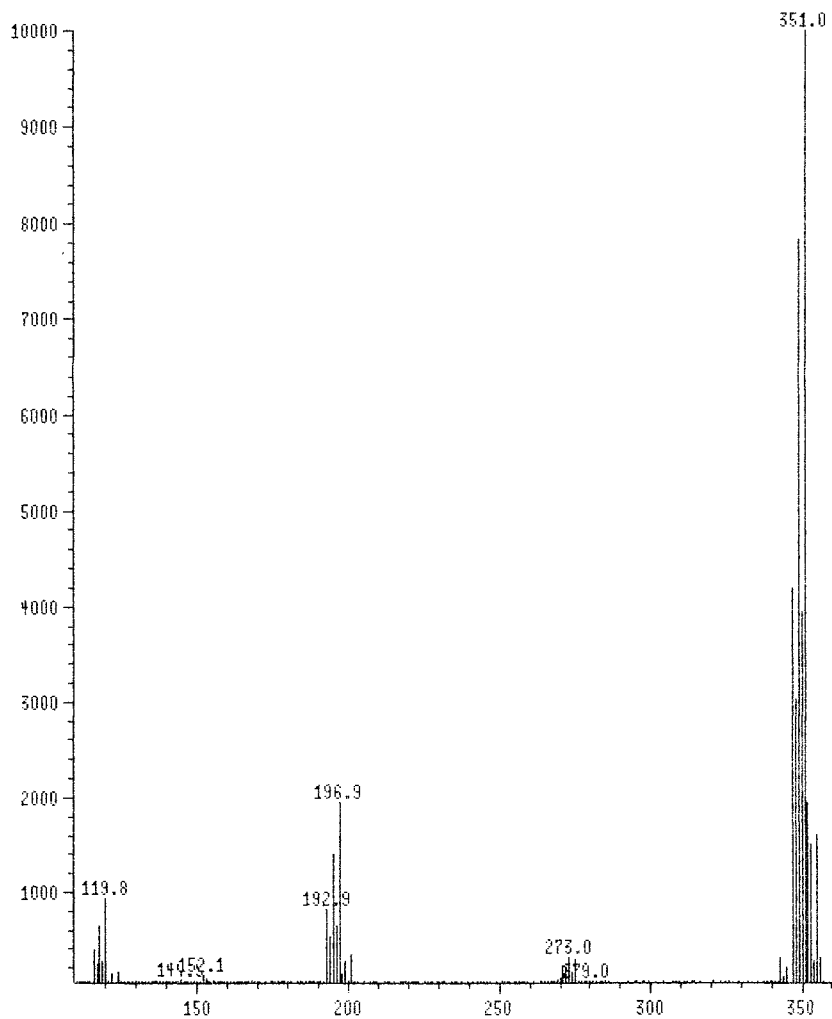


Figure 6: Mass spectrum of TPhT

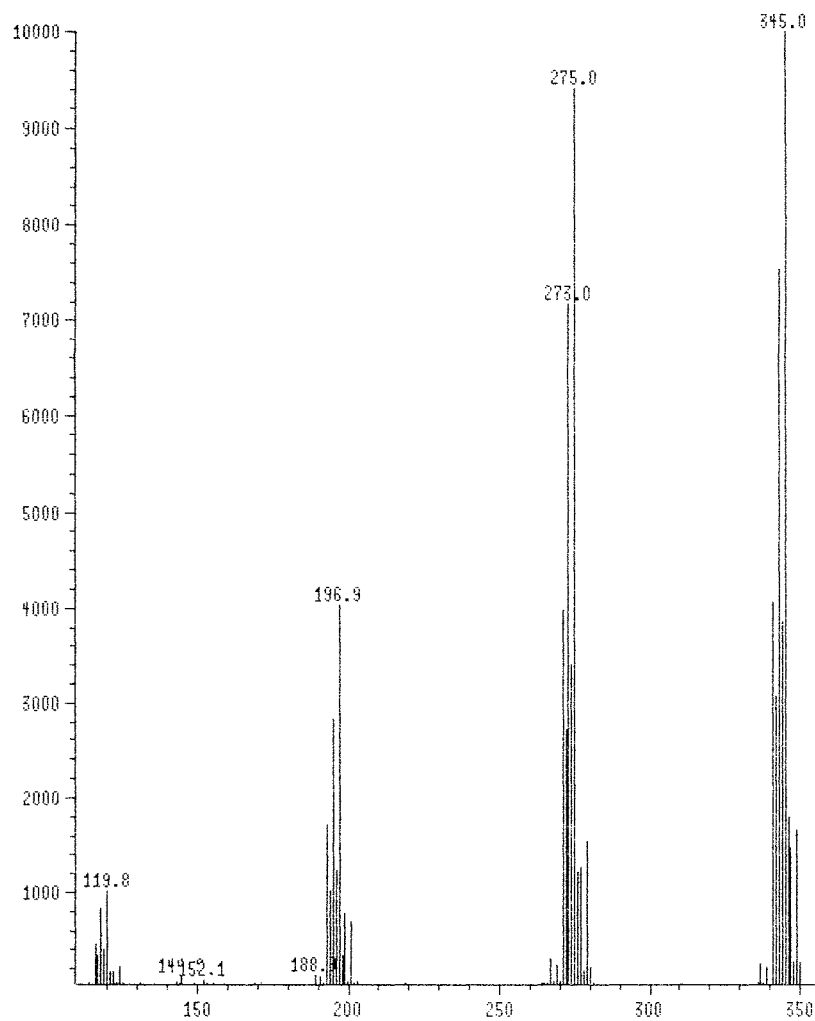


Figure 7: Mass spectrum of DPhT

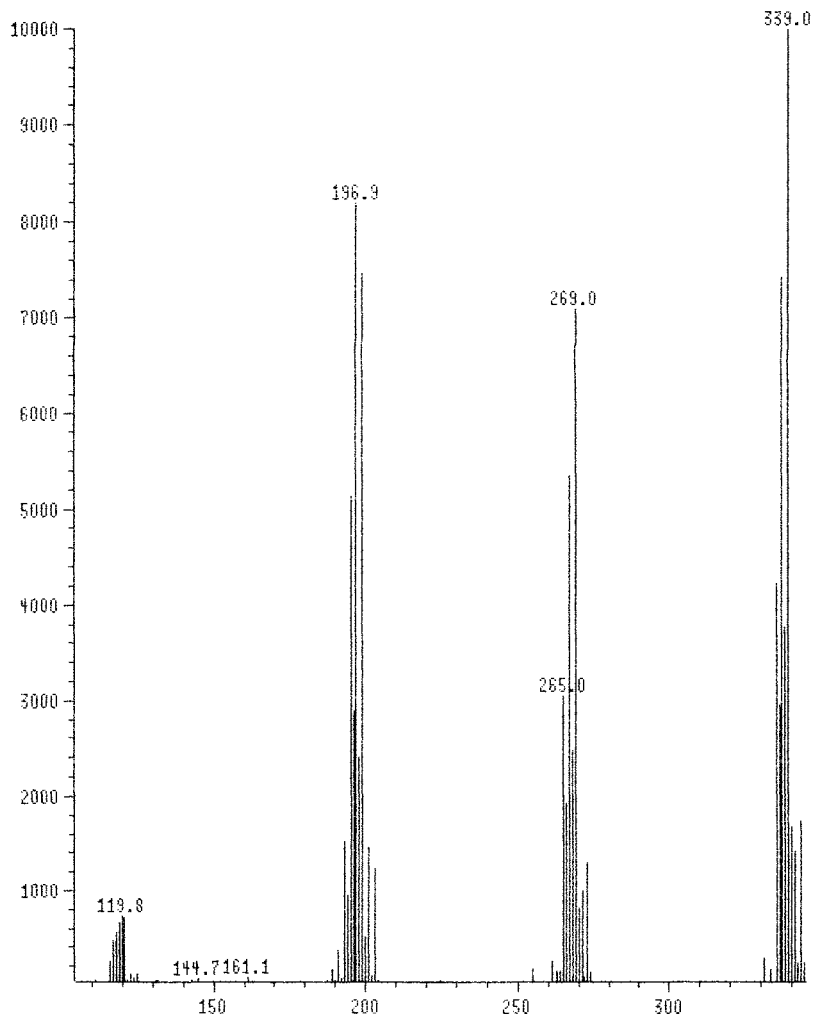


Figure 8: Mass spectrum of MPhT

17.3 Analytical methods

In recent years procedures for butyltins and phenyltins analyses in water, sediments and biological samples were studied on the basis of the literature and optimized in our laboratory. These procedures were frequently tested by using of reference materials, when available, by spiking experiments on real samples and participating to european intercomparison and certification exercises. In this paragraph these procedures are described in detail together with comments on specific topics or hints and recommendations for critical steps in the determinations.

17.3.1 Reagents and materials

- Organic solvents: pesticide analysis grade;
- Tropolone (2-hydroxy-cycloheptatrienone);
- n-Pentyl magnesium bromide 2 mol.l⁻¹ in diethylether;
- Nitric acid;
- Sulphuric acid;
- Potassium dichromate;
- Silica gel: Davison 923 type (100-200 mesh) activated at 180°C overnight.
- Anhydrous sodium sulphate treated at 550°C for 6h before use;
- Tributyltin chloride (TBT); Dibutyltin chloride (DBT), Monobutyltin chloride (MBT); Triphenyltin chloride (TPhT); Diphenyltin chloride (DPhT); Monophenyltin chloride (MPhT);
- Tripropyltin chloride (TPrT);
- SPE LC18 extraction tubes;
- Carboxpack B 80-120 mesh;
- Sn(IV) calibrant solution for GFAAS
- Common laboratory glassware
- Glass reaction vials (8 ml and 15 ml volume)
- Rotary evaporator with vacuum and thermostated bath
- Nitrogen gas blowdown apparatus
- Efficient fume hoods

The purity of commercially available tin compounds is usually based on total tin content and they should be checked for the presence of degradation products with GC-FPD and/or GC/MS after Grignard derivatization. The preparation of calibrant solutions of pentylated organotins following the guidelines given by Ståb *et al.* [72] are strongly recommended, due to the absence of pure standard compounds.

Organotin chlorides stock solutions are prepared gravimetrically in methanol at about 1 mg. ml⁻¹ (as Sn). All data in the text are expressed on the basis of tin) and diluted 1000-fold to give the working standard solutions. When stored refrigerated in the dark, stock solutions are stable for at least 3 months and the working solutions at least for 1 month, but the latter are renewed weekly.

17.3.2 Instrumentation

GC/MS analyses were performed on a Hewlett-Packard HP 5890 GC/ HP 5970B MSD system with the following conditions:

- electron impact ionization mode (70 eV);
- carrier gas: helium, 120 kPa head pressure;
- column: HP-5 (methyl-5 % phenylsilicone, 0.20 mm i.d., 0.11 μ m film thickness, 25 m length; Hewlett-Packard);
- temperature program: 80 °C x 2 min, then 10 °C min⁻¹ to 280 °C; injector: splitless, 240 °C;
- transfer line temperature: 280 °C;

SIM (selected ions monitoring) operation with the following program (dwell time was 100 ms for all ions):

Compounds	Starting time (min)	m/z
TPrT	8	277, 275, 273
TBT	10	305, 303, 301
DBT	12	319, 317, 315
MBT	12.9	319, 317, 315
Sn(IV)	13.8	333, 331, 329
MPhT	15	339, 337, 335
DPhT	16.5	345, 343, 341
TPhT	18.2	351, 349, 347

Table 1

The timings reported above are only indicative and should be adapted to the particular instrumental conditions in use.

Peak identification was based on the matching of retention times (± 0.5 %) and isotopic mass ratios (± 20 %) for the diagnostic ions. The relative response factors were controlled by injecting standard mixtures on a regular basis (one injection every 3-4 samples) to follow the tuning conditions of the MS system.

With these chromatographic settings, the limit of detection for TBT, DBT and MBT at a signal-to-noise ratio of 3 is around 8 pg injected. Phenyltins detection is somewhat more sensitive, particularly for TPhT (LOD=1.5 pg), owing to their peculiar fragmentation pattern.

17.3.3 Procedures

17.3.3.1 Sediments and mussels samples

The sample is homogenized and freeze-dried before extraction. 50-500 ng of TPrT, as internal standard (see 17.3.3.3a), are added to 100-500 mg of sample as methanolic solution before extraction, allowing 30 minutes for equilibration. Longer equilibration times, up to 16 hours, do not affect absolute recovery of TPrT. The sample is placed in a Pyrex vial and 15 ml of tropolone 0.05 % in methanol and 1 ml of concentrated HCl are sequentially added (see 17.3.3.3.b). The vial is put in an ultrasonic bath at a water temperature lower than 40 °C and left under sonication for 15 minutes (see 17.3.3.3.c). The vial is then transferred for centrifugation at 3000 rpm for 10 minutes. The supernatant is transferred in a 250 ml separatory funnel filled with 150 ml of a 10 % NaCl solution (see 17.3.3.3.d) and the extraction procedure is repeated (see 17.3.3.3.e). After the second supernatant is transferred in the separatory funnel, liquid-liquid partitioning is performed twice with 15-20 ml of CH₂Cl₂. The methylene chloride phases are collected through anhydrous sodium sulphate. After washing the sulphate with 1-2 ml of CH₂Cl₂, the extract is added of 1 mL of isooctane and the volume is approximately reduced to 5 ml in a rotary evaporator at a bath temperature lower than 40 °C and under moderate vacuum. The concentrated extract is transferred to a 15 ml vial and lead to almost dryness under moderate flow of nitrogen, operating solvent exchange (methylene chloride to isooctane) (see 17.3.3.3f). 1 ml of 2 mol.l⁻¹ ethereal solution of pentylmagnesium bromide (see 17.3.3.3g) is added and the vial is put under sonication for 1 minute and then in a 50 °C water bath under mechanical agitation; the reaction is allowed to proceed at least for 30 minutes (see 17.3.3.3h). 1 ml of hexane is added and the vial is put in a beaker half-filled with cold water. 2 ml of distilled water are carefully added drop by drop and then 6-7 ml of H₂SO₄ 1 mol.l⁻¹ are added too (see 17.3.3.3.i). Derivatized organotins are extracted with 2-3 ml of hexane; the extraction is repeated twice. The organic phase is put in a vial and concentrated under moderate flow of nitrogen to ca. 0.5 ml. The extract is transferred on top of a silica-gel column (3 g in a glass column 30 cm length and 8 mm as internal diameter) previously wet with 0.5 ml of hexane/benzene 1:1. Hexane/benzene 1:1 mixture is passed through the column until 5 mL are collected in a vial (see 17.3.3.3l). Finally, the solution is concentrated under moderate flow of nitrogen to ca. 0.5 ml. 1 ml is injected for GC-MS determination.

17.3.3.1.1 Recovery tests from sediment samples

Recovery tests from sediment samples were carried out by spiking freeze-dried uncontaminated sediments collected in the open Adriatic Sea. Organotins were added as solutions in methanol to the sediments previously wetted with distilled water. After the addition, sediments were shaken at least for 30 minutes and allowed to equilibrate overnight. Sediments were spiked at two different concentration level in the ranges 140-160 ng.g⁻¹ and 880-1000 ng.g⁻¹. Results, as average of five independent experiments for both the concentration level, are shown in Table 2. As it can be seen recoveries were always satisfactory even if for monosubstituted compounds they were slightly lower than for di- and trisubstituted ones.

Compound	Spiked amount (ng Sn)	Recovery (%)	Spiked amount (ng Sn)	Recovery (%)
TBT	80	91 ± 10	480	94 ± 7
DBT	83	89 ± 12	496	88 ± 11
MBT	75	80 ± 13	450	85 ± 11
TPhT	75	92 ± 11	451	90 ± 9
DPhT	79	87 ± 15	472	91 ± 12
MPhT	74	78 ± 16	442	81 ± 13

Table 2: Recoveries of organotin compounds from 500 mg of spiked sediments. Results are the average of five different experiments. (Ref. [68])

17.3.3.1.2 Recovery tests from mussel samples

Recovery tests on biological materials are difficult to be performed because reference material are not still commercially available (except the NIES - Japanese National Institute for Environmental Sciences - fish tissue certified for TBT). Moreover, uncontaminated samples are rarely collected. Freeze dried mussel samples were analyzed before and after spiking and the recoveries were calculated with respect to the sum of the concentrations of the incurred compounds and the spikes. Spiking procedure was the same as for sediments. Results, as average of five independent experiments, are shown in Table 3. As for sediments, recoveries of monosubstituted compounds were lower than for the other ones. Anyway, recoveries were always higher than 80 % for each organotin compound tested.

Compound	non-spiked (ng Sn)	spiked amount (ng Sn)	found (ng Sn)	recovery (%)
TBT	185 ± 24	160	314 ± 27	91 ± 9
DBT	61 ± 10	165	201 ± 23	89 ± 11
MBT	80 ± 13	150	195 ± 29	85 ± 15
TPhT	nd	150	138 ± 13	92 ± 9
DPhT	nd	157	133 ± 18	85 ± 14
MPhT	nd	147	120 ± 20	82 ± 17

Table 3: Recoveries of organotin compounds from 500 mg of non-spiked and spiked mussels. Results are the average of five different experiments. (Ref. [68])

17.3.3.2 *Water samples*

Filtration of samples is usually performed to discriminate between dissolved and adsorbed fractions. As the toxicological data of organotin compounds and the Environmental Quality Target (EQT) are generally referred to the concentration in the dissolved phase, such a procedure is widely accepted to provide information about the contamination levels. Furthermore, analytical results on non-filtered samples are not easily comparable due to the variety of extraction techniques used for organotin analysis. However, the strong tendency of TBT to accumulate onto suspended matter can lead to underestimate the contamination of the site [38]. In fact, depending on the particular site, the concentration of adsorbed TBT can be equal and in some cases higher than in dissolved phase. Moreover, the contribution of adsorbed TBT in bioaccumulation and toxic effects towards marine organisms cannot be neglected. It is therefore recommended to determine both the concentration of dissolved TBT, after filtration of the samples, and of adsorbed TBT, by analyzing the filters.

The procedure for the determination of organotins adsorbed onto particulate matter is the same as for sediments and mussels.

Usually, a liquid-liquid extraction is employed for water samples due to high performances such as very good recoveries and high enrichment factors. However, it is difficult to be performed in the field and on oceanographic ships during sampling campaigns. In these cases, samples are usually stored and the extraction is performed later. Nevertheless, during sample storage, analytes losses and degradation processes could occur altering butyltins speciation in the samples. Recently, liquid solid extraction (LSE) of TBT [81,113,114] and butyltins [88] from water samples has been successfully applied.

17.3.3.2.1 *Liquid/liquid extraction*

The sample (1 l) is filtered through a 0.45 mm glass fiber filter in order to evaluate the dissolved organotin concentrations.

10-100 ng of TPrT standard solution in methanol are added, depending on the expected contamination level of the sample, and allowed to equilibrate for 15 min with occasional agitation. The sample pH is adjusted to 2 in order to improve the extraction efficiency of the monosubstituted species (see 17.3.3.3b) and the extraction is performed in a separatory funnel with at least 2 aliquots (30 ml) (see 17.3.3.3d) of a 0.03 % tropolone solution in dichloromethane, collecting the organic phases through anhydrous sodium sulphate. The procedure is then the same as in 17.3.3.1.

17.3.3.2.1.1 *Recovery tests*

Certified Reference Materials are not so far available for organotins in water samples; so recoveries from water samples have to be calculated by carrying out spiking experiments. The recovery tests for water samples were performed on uncontaminated filtered sea water. The sample was divided into 3 aliquots. The first aliquot was analyzed and the concentrations of butyltins (TBT, DBT and MBT) and phenyltins (TPhT, DPhT and MPhT) resulted to be below 2 ng.l^{-1} . The other subsamples were spiked with 20 ng.l^{-1} and 200 ng.l^{-1} of each organotin compound, respectively. After spiking, the samples were allowed to equilibrate under mechanical agitation for 2 hours. Recoveries were always higher than 90% for both the tested concentrations and for the six compounds (Table 4).

Compound	Recovery (%) at 20 ng.l ⁻¹	Recovery (%) at 200 ng.l ⁻¹
TBT	102 ± 6	102 ± 4
DBT	104 ± 6	100 ± 4
MBT	97 ± 8	95 ± 10
TPhT	93 ± 9	91 ± 9
DPhT	94 ± 8	91 ± 8
MPhT	91 ± 10	92 ± 13

Table 4: Recoveries of organotin compounds from spiked sea water samples by liquid-liquid extraction. Results are the average of six different experiments. (Ref. [68])

17.3.3.2.2 Solid phase extraction

The complete study is reported elsewhere [88]. The adsorption capacity of solid phases, the recovery and the enrichment factors obtainable with this kind of extraction were carefully evaluated. Adsorption tests were carried out with TBT, DBT, MBT and inorganic tin (Sn IV) respectively. Phenyltins were not systematically tested. On each solid phase (Carbopack, LC 8 and LC 18), inorganic tin was not retained at all; while the retention of organic tin was excellent. In fact, butyltins were completely retained on Carbopack up to 1 mg and on LC 8 and LC 18 up to 6 mg of butyltin compound per gram of solid phase.

Elution tests were carried out for TBT, DBT and MBT with five eluting agents (methanol/tropolone, methanol, dichloromethane, hexane and diethylether). The results obtained on Carbopack and on LC 18 are shown in Table 5; the results are the average of five different replicates and the final measurements was performed by GFAAS. As it can be seen, both for Carbopack and LC 18, methanol/tropolone was able to quantitatively elute all the butyltin compounds at the same time, with high efficiency. Similar results were obtained on LC 8 but, in this case, larger volume of eluants (about 10 ml) were needed.

	methanol/ tropolone	methanol	dichloro- methane	hexane	diethylether
TBT	105±7 94±7	105±4 96±8	27±8 <10	<10 0	0 0
DBT	102±9 101±5	0 0	0 0	0 0	0 0
MBT	107±5 89±4	0 0	0 0	0 0	0 0

Table 5: Butyltins elution test from Carbopack and LC 18. Results, expressed as percentage, are the average of five different experiments. The first column in each row indicates the results obtained with carbopack, whereas the second column gives the results obtained with LC 18 (Ref. [88])

17.3.3.2.2.1 Recovery tests

Recovery tests were carried out on deionized water and sea water. LC18 pre-packed tubes (500 mg) and Carboxpack B (100 mg) were tested. Both tube types were pre-treated sequentially with 10 ml of methanol and 10 ml of distilled water, avoiding the drying of the adsorbent bed. The sample volumes to be extracted were selected on the basis of acceptable sample flowrates (for LC 18 the manufacturer recommended flowrate of 5 - 10 ml.min⁻¹ was found to be satisfactory; for Carboxpack B 10 ml.min⁻¹ was used, even if higher flowrates can be accommodated by this solid phase). TPrT as internal standard is added to the sample as in the liquid/liquid extraction procedure. Adsorbent bed must be vacuum dried before solvent elution.

Different aliquots of both deionized and sea water were spiked with 40 ng.l⁻¹ and 200 ng.l⁻¹ of each butyltin species, respectively. After spiking the samples were allowed to equilibrate under mechanical agitation for 2 hours. 1 l samples can be extracted without losses using Carboxpack B, whereas volumes larger than 250 ml cannot be extracted by LC18 columns without significant losses (e.g. recovery from 500 ml sea water sample spiked at 200 ng.l⁻¹ was 78 %). Differences between recoveries from sea water and deionized water are not statistically significant. Results obtained from spiked sea water samples extracted with LC 18 and Carboxpack are shown in Table 6.

Compound	LC 18 (250 ml) 40 ng.l ⁻¹	LC 18 (250 ml) 200 ng.l ⁻¹	Carboxpack (250 ml) 40 ng.l ⁻¹	Carboxpack (250 ml) 200 ng.l ⁻¹	Carboxpack (1 l) 40 ng.l ⁻¹	Carboxpack (1 l) 200 ng.l ⁻¹
TBT	96	96	95	103	99	102
DBT	90	90	88	99	96	98
MBT	88	90	87	94	90	94

Table 6: Recoveries of butyltin compounds from spiked sea water samples by liquid-solid extraction. Results are the average of two different experiments. (Ref. [68])

17.3.3.3 Remarks on the procedures.

As a general rule for trace analyses, chemical reagents should be of the highest purity available and should be individually tested for interferences and quality variations from batch to batch. Further purification steps are sometimes necessary in organotin analyses: in particular, dichloromethane pre-extraction of chloridric acid (often contaminated by MBT) was needed. Thermal treatment of other reagents (sodium sulphate, silica gel) is highly recommended, particularly if purchased in plastic containers.

Grignard reagents, as previously mentioned, are often contaminated by butyltins, the contamination varying among different batches [115]. Values of e.g. TBT contamination ranged from 4 to 15 µg.l⁻¹. Custom synthesis of Grignard reagents is therefore recommended if very low contaminated samples are to be analyzed.

- a) Tripropyltin was used as internal standard for quantitative analysis, being a compound that closely matches the most environmentally relevant compounds (TBT and TPhT) to be determined and because it was neither detected in natural samples nor its presence is expected, lacking a widespread use.
- b) The extraction of organotin compounds is to be performed in acidified medium to enhance the recovery of di- and particularly monosubstituted species [63,66]. Acidity acts therefore synergistically with the complexing agent, tropolone. In water samples, acidification is needed to break the organotin aquocomplexes, to avoid possible binding to container walls or dissolved matter (*e.g.* humic substances), *etc.*. It also helps to reduce emulsions. In solid samples and particularly in sediments, acidification destroys the carbonates matrix, helps the release of mono- and disubstituted organotins from complexes with sulphur compounds, humics, *etc.*. The use of a low concentration of strong acid is a compromise between effective matrix destruction and the need to avoid speciation changes: phenyltins are very sensitive to pH extremes. Tropolone is a well suited ligand for tin. It is effective also in acidic solutions unlike *e.g.* 8-hydroxy-quinoline and does not give side effects (obnoxious smell) as APDC. It is therefore the ligand of our choice in the analysis of organotins in environmental matrices. Tropolone forms well stable complexes with mono- and diorganotins while triorganotins are not complexed at all at concentrations of environmental concern [116].
- c) Ultrasonic bath and rotary evaporator bath temperatures are kept below 40°C to avoid risks of organotins degradation (and consequently speciation changes) or losses by volatilization (care must be taken to avoid the complete drying of the extracts under vacuum).
- d) The addition of sodium chloride is necessary to reduce emulsion problems and to enhance back-extraction efficiency from the aqueous solution to methylene chloride.
- e) Sequential extraction experiments on sediments and mussels showed that more than 90 % of extracted organotins were in the first extracting aliquot. Two extraction steps are a compromise between speed of analysis and efficiency.
- f) Isooctane is added to the extract as a keeper to reduce the risks of evaporative losses.
Solvent exchange is needed because dichloromethane is not compatible with Grignard reagents.
- g) CAUTION: Grignard reagents are hazardous chemicals. They violently react with acids, water, alcohols, ketones, *etc.* and should be handled with extreme care by well trained personnel and using opportune safety precautions (gloves, glasses, *etc.*).

- h) Sample extracts from sediments or mussels contain high amounts of coextractants and after addition of the Grignard reagent a precipitate is often found. Sonication of the solution is performed to improve sample-to-reagent contact by partial resolubilization of the precipitate.
Although Grignard derivatization is rapid and effective also at room temperature on standard solutions, higher temperatures and time of reaction are necessary to improve recovery from dirty samples. The reaction conditions used do not affect speciation, as experimentally observed.
- i) Excess Grignard reagent must be destroyed before cleanup and analysis. Extreme care must be paid by the operators in this step. The drop by drop careful addition of water, keeping the vial in a cold water bath, is necessary to reduce the risks of a too violent reaction. The addition of hexane (or isooctane) before this step reduces the risks of evaporation losses.
- l) Recovery tests from cleanup were performed using derivatized standard of butyltins, phenyltins and tripropyltin. Recoveries higher than 90 % were found for all organotins using 5 ml of hexane/benzene.

Derivatized organotins are very stable compounds. Non derivatized organotins are subject to easy decomposition, particularly when they are in solution at room (or higher) temperature as usually happens during the procedure above described. Thus, it is necessary to ensure the shortest time between extraction and derivatization to minimize the risk of decomposition. This is particularly true for phenyltins: a degradation of TPhT directly to MPHT has been sometimes observed as a consequence of a delay in the derivatization.

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18.

Development of supercritical fluid extraction procedures for the determination of organotin compounds in sediment

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Analytical-scale supercritical fluid extraction (SFE) is one of the most relevant emerging technologies for sample preparation and was introduced at the end of the last decade. Until now, the potential of this technique was well proven in the case of organic contaminants due to the unique properties of supercritical fluids [1-3]. However, its application to organometallic extraction in speciation studies has hardly been investigated. Apparently, the lack of inert supercritical fluids with enough solubility for their extraction from inorganic matrices such as sediments, where they can be strongly bound onto polymeric materials (*i.e.* humic and fulvic substances)[4], may explain this fact.

This contribution summarizes the development of several (SFE) approaches for the determination of organotin compounds in sediments and their comparison with another extraction procedure based on atmospheric-pressure liquid extraction (APE).

The aim of the application of SFE for tin speciation studies is to improve the extraction efficiency and selectivity of existing extraction procedures. In fact, reported recoveries of organotin compounds in the literature are usually overestimated because they have been determined from spiked samples, while naturally incurred organotin compounds in sediments display stronger binding behaviour onto the matrix [5]. In addition, the lack of reference materials with certified values for all organotin compounds of environmental relevance renders the method development difficult. In this respect, the use of a well validated SFE procedure is of great interest in the development of certified reference materials, because extraction is based on a different principle from classical extraction methods.

18.1 Fundamental Aspects

The potential of analytical-scale SFE lies, mostly, in the unique properties of supercritical fluids (SFs). The higher diffusion coefficient and lower viscosity of SFs in comparison with liquids enable faster extraction rates (Table 1). Moreover, the critical surface tension, equal to zero, is responsible for enhanced mass-transfer from porous matrices to the fluid leading to higher recoveries. Another unique property of supercritical fluids is the dependency of solubility with density which usually increases with pressure and decreases with temperature. Below a threshold density, analytes are insoluble in the supercritical fluid. Consequently, some selectivity can be gained by tuning the density of the supercritical fluid used in the extraction.

Extraction selectivity is an aspect of primary interest in case of tin speciation from complex matrices like sediments, because co-extractants may inhibit derivatization reactions or yield interferences in the detection step [6,7].

Table 1: Order of magnitude of density (ρ), viscosity (η) and diffusion coefficient (D) according to the fluid states

Fluid state	ρ (g cm ⁻³)	η (Pa. s)	D (m ² .s ⁻¹)
Gaseous			
(1 atm, 15-30 °C)	(0.6-2).10 ⁻³	(1-3).10 ⁻⁵	(0.1-0.4).10 ⁻⁴
Supercritical			
Tc, Pc	0.2-0.5	(1-3).10 ⁻⁵	0.7.10 ⁻⁷
Tc, 4Pc	0.4-0.9	(3-9).10 ⁻⁵	0.1.10 ⁻⁷
Liquid			
(1 atm, 15-30 °C)	0.6-1.6	(0.2-3).10 ⁻³	(0.2-2).10 ⁻⁹

Tc: critical temperature; Pc: critical pressure. Adapted from [8]

Although a large variety of liquids have critical constants (Pc, Tc and ρ_c) compatible with the available analytical instrumentation (Table 2), cost, toxicity, safety and environmental considerations, restrict the choice of supercritical fluids to CO₂ and N₂O. However, the extraction of ionic compounds (i.e. organotins) is not feasible in the ionic form because of their lack of solubility in CO₂.

Table 2: Selected properties of supercritical fluids (SF) used in SFE

SF	T _c (°C)	P _c (atm)	ρ _c (g cm ⁻³)	δ _{SFC} (cal ^{-1/2} cm ^{-3/2})
ethene	9.2	49.7	0.217	5.8
xenon	16.6	57.6	1.113	6.1
CO ₂	31.0	72.9	0.466	7.5
N ₂ O	36.4	71.5	0.452	7.2
SF ₆	45.5	37.1	0.738	5.5
NH ₃	132.4	111.3	0.235	9.3
n-C ₅ H ₁₂	196.5	33.3	0.237	5.1

T_c: critical temperature; P_c: critical pressure, ρ_c: density in the critical point and δ_{SFC}: solubility parameter of supercritical fluids.

In order to circumvent that limitation, supercritical fluid modifiers are necessary to enhance their solubility in CO₂. Among the modifiers potentially of interest in SFE, methanol is the most commonly used due to its high dipolar moment and its favourable miscibility in supercritical CO₂ [9]. Another approach to enhance the solubility of polar contaminants in supercritical CO₂ is to perform a chemical reaction during the extraction by blocking the polar groups of the analyte with less polar substituents (*e.g.* alkyl, acetates) [10,11]. This approach, however, has not been fully explored for the extraction of organometallic compounds.

In the case of ionic species, the formation of counter-ions has been successfully tested for the extraction of anionic surfactants from sewage sludge [12] but in case of di- and trialkyltins, it did not enhance the recovery from top soil samples when NaCl was used [13].

The stability of metal chelates (*i.e.* bis(trifluorethyl) dithiocarbamate) in supercritical fluid conditions enables the chromatographic separation by capillary supercritical chromatography (SFC) of metal ions (As³⁺, Bi³⁺, Co³⁺, Fe³⁺, Hg²⁺, Sb²⁺ and Zn²⁺) [14]. However, a similar approach has not yet been evaluated in the case of SFE; in fact only one report shows that sodium diethyldithiocarbamate can enhance the extraction recovery of spiked organotins from top soil samples [13] but this procedure has not been tested in natural matrices.

18.1.1 SFE Instrumentation

Several instrumental set-up and analytical-scale SFE apparatus are commercially available. Figure 1A gives an overview of off- and on-line SFE instrumentation based on extract collection or direct transfer to a chromatographic system. The fluid in the liquid state and the modifier are delivered by high-pressure syringe or reciprocating pumps. After extractant agent mixing, the resulting binary mixture is transferred to the extraction cell, which is located in a thermostated chamber. Following a back-pressure regulation system (flow-restrictor or valve) the extract is collected within a solvent or

trapped into an adsorbent (*e.g.* ODS, Tenax, florisil, silica, alumina, Carboxypack), which can be refrigerated to favour the trapping efficiency of the most volatile components. After the completion of the extraction period, the recovered extract is eluted with a solvent using an HPLC pump. If a second pump is not available, the modifier is added directly into the extraction cell.

In this work, dual high-pressure syringe pumps have been used and consequently, the primary fluid and the modifier are mixed upstream of the extraction cell.

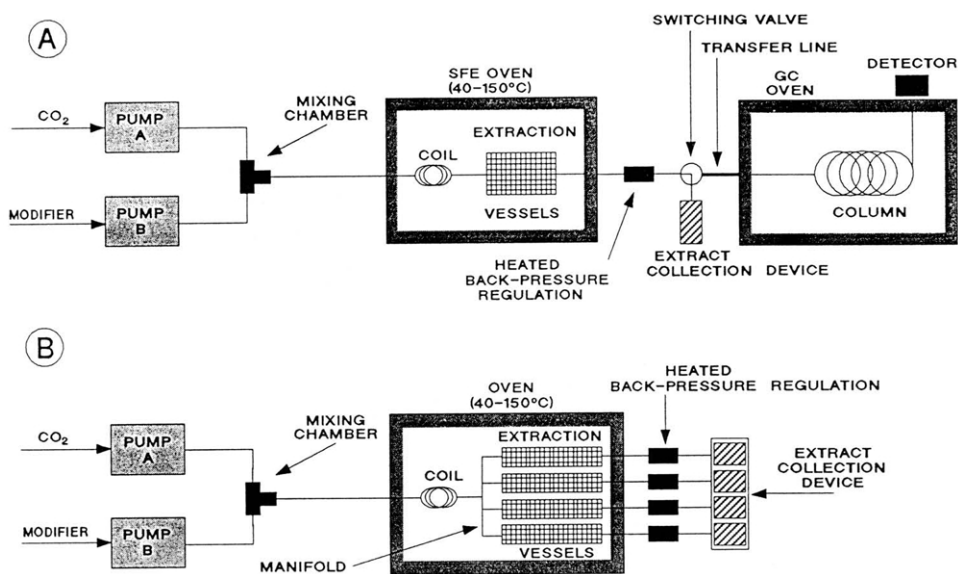


Figure 1: Analytical-scale SFE instrumentation

By means of a valving system, extraction can be performed in dynamic or static modes, depending whether the supercritical fluid continuously sweeps the extraction cell or not. Generally, a combination of both extraction modes yields higher extraction recoveries. In the static extraction mode, it is not necessary to use a back-pressure regulation system and the extraction cell effluent is connected to the collection vial by means of stainless steel tubing; in this way, restrictor plugging may be avoided [15] which is one of the most faced problems in SFE using fixed flow-restrictors.

In order to increase the sample throughput, parallel cell configuration or automated sequential extraction have been introduced into the market, enabling higher productivity to be achieved (Fig. 1B). Although off-line SFE is the most widely used approach owing to its higher flexibility, the on-line approach can be useful for the analysis of samples containing very low concentrations of the analytes of interest. In this way the sensitivity is increased by several orders of magnitude, provided that whole extract is transferred to the analytical column.

The main limitation of this approach is the need of extraction conditions selective enough to avoid matrix interferences at the chromatographic step. In order to solve this problem, several SFE-GC interfaces have been developed using modified vaporizing GC injectors (*i.e.* split-splitless, PTV), which enable the selective transfer of volatile compounds. In this work, preliminary results obtained with the on-column SFE-GC interface will be reported where the whole SFE extract is transferred to a room temperature retention gap by means of a linear restrictor.

18.1.2 Speciation Techniques

Although HPLC chromatographic techniques have been used in tin speciation studies [16,17], GC coupled to different detection systems has been most generally used in the past few years. Table 3 shows a comparison of the sensitivity obtained with different detection systems. Among them, GC-FPD using a 610 nm filter is a technique well suited for routine analysis due to its robustness and simplicity. However, volatile derivatives are required for tin speciation by GC alkylated derivatives are usually preferred over hydride counterparts due to their higher stability.

Attempts to determine free halides of organotins by GC-FPD have not been completely successful because a hydrochloric acid treatment of the analytical column is required [18,19]. Capillary columns coated with non-polar stationary phases are the most widely used [20].

Table 3: Sensitivity of different detection systems coupled to GC techniques for the determination of butyltin compounds.

Analytical column	Derivative	Detection system	LOD ^a (pg as Sn)	Reference
Capillary	Pentyl	FPD-QSIL ^b	2-3	[21]
Megabore	Pentyl	QFAAS	17-37	[22]
Megabore	Pentyl	GFAAS	33-71	[22]
Capillary	Pentyl	AES	0.1-0.15	[22]
Capillary	Methyl	SF-FPD-GPL ^c	9.7-40.1	[23]
Capillary	Methyl	MS-SIMS	3.5-4.2	[23]
Packed	Ethyl	ICP-AES	25	[24]

^aS/N = 3

^bQuartz surface induced luminescence

^cSingle-flame FPD gas phase luminescence.

In order to circumvent the lack of stability of organotin halides arising in GC conditions, Dachs and Bayona [25] have optimized the FPD response of those compounds in supercritical fluid chromatography (SFC). They found that compound degradation is minimized at low temperatures (50 °C) but still monobutyl- and monophenyltin could not be eluted using density programming of carbon dioxide. Similar results have been obtained using packed column SFC but in this case a modifier was used (HCOOH) [26]. Despite detection limits which are higher (40-330 pg as tin) than those obtained in GC conditions for the same compounds, analysis time and dynamic range are comparable.

18.2 Development of supercritical fluid extraction procedures

18.2.1 Variables in SFE

Many variables may have a quantitative effect on the SFE efficiency (Table 4) [27], some of them being dependent on the sample nature (matrix, particle size, sample size), analyte characteristics (analyte type, mode of analyte accumulation). In order to improve both inter- and intralaboratory reproducibility, all of these parameters must be considered during the method development. The kind of interaction between analyte and matrix is another aspect that should be considered. Organotin compounds can adopt a variety of counter-ions in seawater (e.g. CO_3^{2-} , Cl^- , OH^- , S^{2-}) and in sediments [28] and they could be bound onto the polymeric organic matter (humic and fulvic substances) [4].

Sample -size is an important point to be considered. Usually, it should be as small as possible depending upon the concentration levels of analyte and the homogeneity of the sample. It is also important to avoid dead volumes in the extraction cell that could lead to lower recoveries. Dead volumes in the extraction cell could be filled up with an inert material (e.g. silylated glass beads) or using an extraction cell with a smaller volume.

Table 4: Variables which could affect SFE efficiencies

Analyte properties (polarity, molecular weight)
Extraction cell agitation
Extraction cell dead volume
Extraction cell geometry (length/wide ratio)
Extraction cell size
Extraction fluid (polarity, solubility parameter)
Flow restrictor type (fix/variable)
Fluid flow-rate (constant/variable)
Fluid modifiers (solvents,derivatizing agents)
Mode of analyte accumulation
Modifier concentrations
Pressure (density)
Sample characteristics (humidity, pH, particle size, amount)
Temperature (density)

Adapted from [27]

Usually, other variables should be considered during the method development and validation, *e.g.* extraction temperature, pressure, fluid composition and volume. Extraction temperature should be as high as possible because it increases the diffusion coefficient, therefore enhancing the mass transfer which is usually the limiting factor of the SFE kinetics [29]. Extraction pressure is another key variable; the solubility of supercritical fluids increases with pressure and it should be maintained as low as possible in order to keep the extraction selectivity. Similar considerations can be made in the case of supercritical fluid composition *i.e.* using the lowest solubility parameter as possible, provided that a quantitative extraction of the analyte is achieved. Finally, the volume of supercritical fluid should be at least from five to ten times higher than the volume of the extraction cell in the dynamic SFE extraction mode. If higher volumes of supercritical fluid are used, they do not significantly improve the recoveries obtained.

Taking all of these variables into account, SFE methods can be reproduced on varying types of instrumentation with increasing confidence [30].

18.2.2 Method development

The kind of interaction between the analyte and the matrix must be considered in SFE method development. The stronger the interaction, the higher the solvating power needed. Nevertheless, the strength of the interaction between analyte and sample can be matrix dependent and consequently the same extraction procedure can lead to a different extraction efficiencies from sample to sample. Although spiked sediments are useful for method development, the accuracy of SFE extraction procedures should be assessed with different certified reference materials (CRMs), which will be addressed in section 18.2.3.

As mentioned in section 18.1.1, supercritical CO₂ is the most widely used fluid. Unfortunately, a poor tributyltin (TBT) recovery from a spiked sediment was obtained with neat CO₂ in SFE conditions. Extraction yield was comparable to that obtained by high-pressure liquid extraction (HPLC) (21.2%) with liquid carbon dioxide by using a high-pressure Soxhlet apparatus. These results are consistent with a similar solubility of supercritical fluids at high pressures in comparison of liquids. Nevertheless, extraction was accomplished in 1/6 of the extraction time (30 min in SFE *versus* 3h in HPLC). The poor TBT recoveries obtained in both methods using CO₂ could be accounted for by a stronger interaction of analytes with the matrix in comparison to CO₂. A sample pretreatment with hydrochloric acid in order to release the corresponding organotin chlorides led to an enhancement of 5% in the extraction efficiency of TBT. These results are consistent with the satisfactory solubility of organotin chlorides in supercritical CO₂, shown in SFC [25]. Therefore, a SFE method development has been outlined using CO₂ in presence of an organic modifier. Following the SFE, organotin chlorides are derivatized with a Grignard reagent (C₂H₅MgCl) to obtain the corresponding ethylated derivatives, as described elsewhere [32]. In this way, the volatile compounds obtained can be determined by GC-FPD. This analytical procedure will be referred to as sequential supercritical fluid extraction and derivatization (SFE-D) (Figure 2).

The following variables have been considered:

Extractant agent composition. Methanol was chosen as a carbon dioxide modifier considering its miscibility in CO_2 , the reasonable solubility of organotin compounds and its favourable behaviour in SFE [9]. From 5 to 15 % v/v of MeOH was used as modifier. Methanol was doped with hydrochloric acid gas (0.01 mol.l^{-1}) in order to generate the corresponding organotin chlorides, which are more soluble in supercritical CO_2 .

Pressure. A range between 20 and 35 MPa was considered. Higher pressures do not increase the density significantly and, consequently the solubility of the supercritical fluid. Hence they were not considered in the optimization procedure. 20 MPa was considered to be the lowest extractable pressure taking into account the SFC retention volumes of TBT chloride [25].

Temperature. The lowest value must be higher than the critical temperature (T_c) of the fluid, i.e. in the case of neat carbon dioxide higher than 31°C (Table 2). Since it is modified due to addition of methanol, the T_c of the binary fluid will actually be higher [8]. Accordingly, 50°C is considered the lowest practical temperature and 80°C the highest, taking into consideration the restricted thermal stability of monobutyltin and dibutyltin chlorides in supercritical CO_2 [25].

Extraction time. The application of modifiers prevents the real time extraction-monitoring in most of the flame detectors used to monitor the SFE process. Consequently, this variable needs to be included in the optimization procedure (15-30 min).

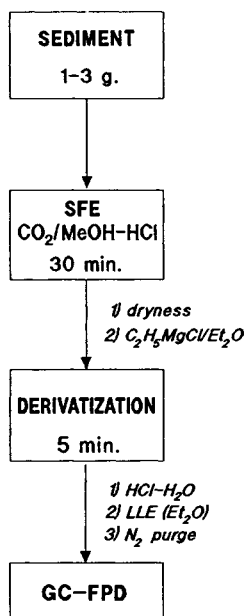
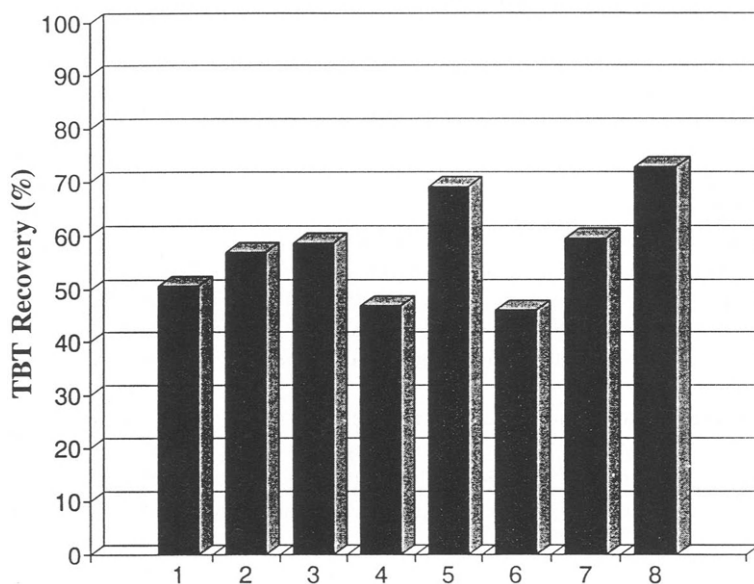


Figure 2: Analytical scheme followed in the SFE combined with a derivatization procedure (SFE-D).

Extraction variables were optimised following a factorial analysis using a fractional approach [33,34]. Response effects were determined by the Yates algorithm [35] which enables the principal effects and the interactions between variables to be determined. Recoveries obtained ranged from 46.0 to 72.8 (Figure 3).



TEMPERATURE	(°C)	50	80	50	80	50	80	50	80
PRESSURE	(MPa)	20	20	35	35	20	20	35	35
EXTR. TIME	(min)	15	15	15	15	30	30	30	30
v/v MeOH	(%)	5	15	15	5	15	5	5	15

Figure 3: Experimental design for the optimization of the TBT SFE in sediments.

Following the statistical treatment, it was shown that the most significant variable was the modifier composition and to a lesser extent the extraction temperature. Further, interactions between variables were not significant. Taking into account this lack of interaction, further optimization was performed in order to improve the extraction efficiency by increasing the methanol content and keeping the temperature constant. The steady increase of extraction efficiency reached a plateau around 15 % of MeOH doped with HCl [31]. The steeper increase of TBT recovery at low concentrations of modifier could be interpreted in terms of a competition between adsorbed organotins on the active sites of the matrix with the modifier. However, at higher concentrations of modifier, this effect was less important and could be attributed to an increase in the mobile phase solubility. Due to practical considerations, however, the extraction temperature had to be increased from 50 to 60 °C to minimize restrictor plugging. Therefore, optimum conditions were as following: $T = 60\text{ }^{\circ}\text{C}$, $P = 35\text{ MPa}$, 20 % of MeOH doped with HCl in CO_2 v/v, $t = 30\text{ min}$ dynamic. Extraction efficiency for TBT, referred to the spiking level, was 82 %, which could be considered satisfactory according to the spiking procedure used to prepare this sample [36]. Figure 4B shows a GC-FPD chromatogram of the extract recovered from a TBT spiked sediment, showing only the presence of low concentrations of DBT, which was also identified in the blank sample, whereas monobutyltin and inorganic tin were not present. The application of the developed SFE hence enabled a quantitative recovery of TBT avoiding its degradation under the extraction conditions used. The lack of interferences found in the GC-FPD trace of the SFE extract isolated from this matrix is remarkable since no clean-up steps were performed.

Taking into account the high recoveries obtained for tetraalkylated organotins with neat CO_2 from spiked soils [13], we have developed another extraction approach for the determination of butyl- and phenyltins in sediments using an *in situ* sequential derivatization and extraction (D-SFE) of the derivatized forms of organotin compounds with CO_2 [37]. The main advantages of this procedure are as following: (i) higher selectivity because no modifiers are needed, (ii) both parent and degradation products could potentially be extracted due to a similar solubility in CO_2 , and (iii) derivatization is performed *in situ*, which allows an easier sample handling (Figure 5).

As it is shown in Figure 5, hexylated derivatives were used because of their higher solubility in CO_2 and lower volatility than their ethylated counterparts, which minimized losses during the extract trapping step. The optimization of the SFE with neat CO_2 from a laboratory spiked sediment has been carried out using an experimental design taking into account three variables (pressure, temperature and extraction volume) [38]. The most significant variables were pressure and temperature. In the most feasible extraction conditions ($P=35\text{ MPa}$, $T=40\text{ }^{\circ}\text{C}$, $t=10\text{ min}$ static and 10 ml dynamic), the extraction efficiency and precision were satisfactory for di-phenyl whereas mono-phenyl exhibited poor extraction efficiency and reproducibility, probably due to a greater interaction of these compounds with the matrix or to lower reactivity with the Grignard reagent.

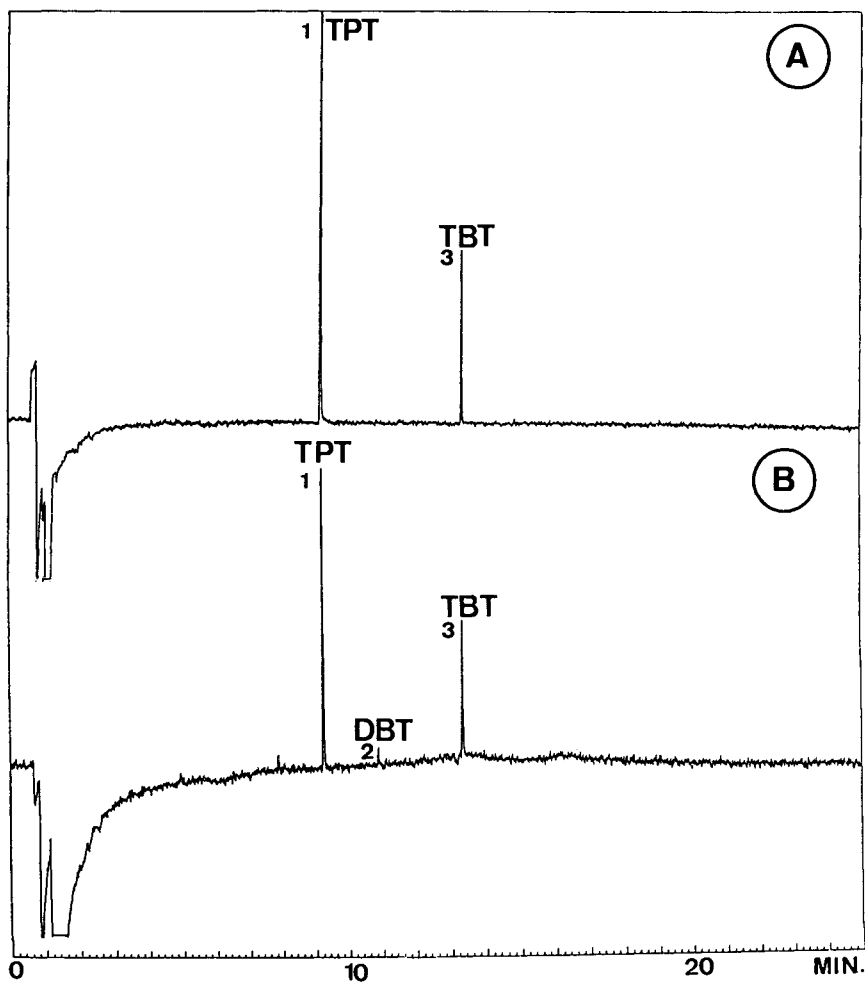


Figure 4: GC-FPD chromatograms of A) standard compounds and B) TBT spiked sediment extracted by the SFE-D procedure. Compound identification is as follows: 1) tripropyltin (internal standard); 2) dibutyltin and 3) tributyltin as ethylated derivatives. Analytical column was a 30 m x 0.25 mm i.d. coated with 0.10 μm of DB-5. Column temperature was held at 35 $^{\circ}\text{C}$ at 8 $^{\circ}\text{C}.\text{min}^{-1}$, keeping the final temperature for 5 min. Hydrogen was used as carrier gas with a flow rate of 50 $\text{cm}.\text{s}^{-1}$. Detector temperature was setup at 225 $^{\circ}\text{C}$.

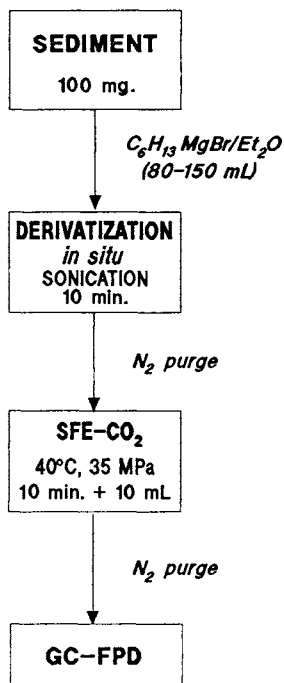


Figure 5: Analytical scheme used in the *in situ* sequential derivatization and extraction (D-SFE).

Several attempts have been made to improve the derivatization reaction yields by *e.g.* increasing reaction time and the amount of Grignard reagent (80-150 μ l) and applying sonication to favour the contact of reagent with the matrix. However, only the latter led to an improvement of the reaction yields. Solvent removal before the SFE is of primary importance in order to obtain clean organic extracts since the organic solvent of the derivatizing agent (Et₂O) may act as a CO₂ modifier enhancing its solubility; this can be easily performed by passing a gentle purge of nitrogen through the extraction cell.

Table 5: Analytical results achieved in the SFE of a spiked sediment by sequential derivatization and extraction approach (D-SFE)

	TBT	DBT	MBT	TPhT	DPhT	MPhT
Recoveries (%)	111	76	15	114	106	40
S.Ds*	2	7	7	10	11	34
R.S.Ds (%)	1.8	9.2	46.6	8.7	10.4	85
L.O.Ds (ng.g ⁻¹)	1.7	1.5	1.8	2.4	2.2	5.8

* Standard deviation of 5 independent replicates.

Further attempts were carried out by pretreating the sediment with several different concentrations of hydrochloric acid but in all the experiments increasing contents of inorganic tin and lower contents of organotins were detected, probably associated with their degradation during this pretreatment. The analysis of the SFE extracts from previously extracted samples did not reveal the presence of significant concentrations of any tin compounds. Furthermore, procedural blanks of the empty extraction cell in presence of the derivatization agent did not reveal the presence of any compound in the retention time window of organotins.

The LOD of the analytical procedure is satisfactory (Table 5) and is comparable to other data reported in the literature (Table 6). Nevertheless, it could be improved by extracting larger amount of sample, concentrating to lower volumes or using larger injections on the GC apparatus. Another way to improve detection limits is to use an on-line SFE-GC and transfer the whole extract to the chromatographic system. Figure 6 shows some preliminary results obtained using this approach. Although it is feasible using the on-column interface, many compounds can be co-extracted with organotins, leading to interferences in the GC-FPD determination. Moreover, if hexylated organotin derivatives are prepared, it is not necessary to use any cryogenic trapping system to focus the chromatographic band. The main drawback of this approach is associated with the transfer of whole analytical extract to the analytical column, which can affect the column performance when environmental samples are extracted. In this case, it is necessary to introduce an on-line clean-up prior to the chromatographic system. In this respect, Pyle and Setty [39] have found that copper is a useful sulphur scavenger from the SFE extracts.

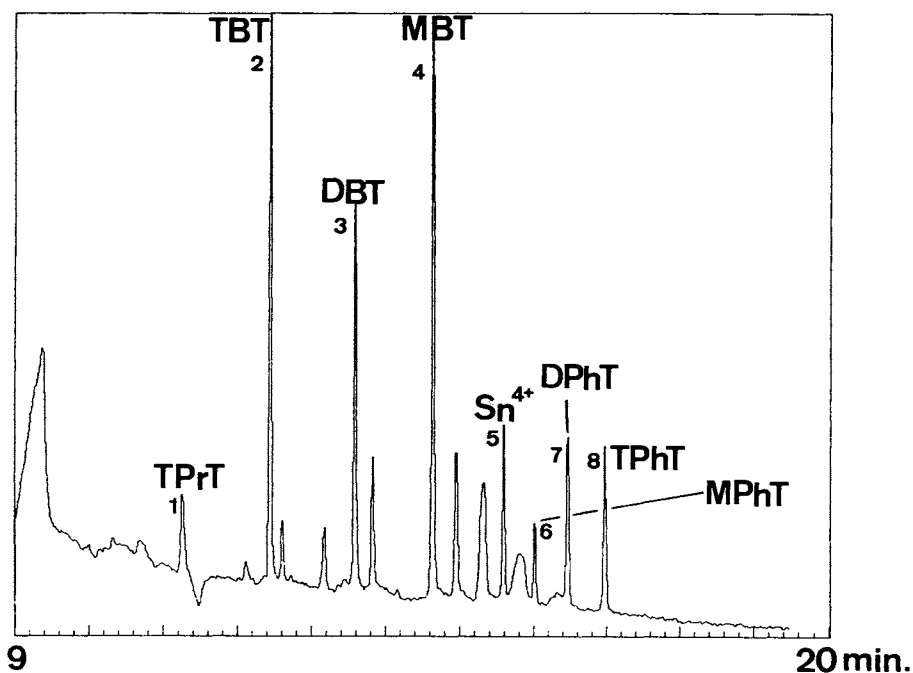


Figure 6: On-line SFE-GC-FPD hexylated organotins obtained by spiking the derivatized compounds in glass beads. The spiking level was in the range 0.5 to 0.84 ng onto 150 mg of glass beads. Compound identification as follows: 1, tetrabutyltin; 2, tributyltin; 3, dibutyltin; 4, monobutyltin; 5, inorganic tin; 6, monophenyltin; 7, diphenyltin; and 8, triphenyltin.

Table 6: LOD and precision of reported analytical procedures for the TBT determination in sediment

Procedures	Recovery (%)	LOD (ng.g ⁻¹ as Sn)	RSD (%)	Ref
Tropolone-C ₆ H ₆ , Bu ₃ PeSn, silica gel, GC-FPD	81	3	25.7	[41]
MeOH-HCl. Reflux. Hydride generation	142	0.2	7(n=4)	[5]
MeOH-HCl, silica-HCl, EtOH-NaBH ₄	93	20-40	2.6	[42]
MeOH-HCl, ethylation, GC-AAS	92	12	5	[40]

18.2.3 Method validation

In order to evaluate the accuracy and reproducibility of both SFE procedures, five independent replicates of a Certified Reference Material (CRM) of sediment certified for its TBT content, PACS-1 (National Research Council, Ottawa, Canada), were performed. Figure 7 shows a characteristic GC-FPD chromatogram where no significant interferences were observed in the determination of these compounds. The analytical results are listed at Table 7. The reproducibility was considered to be satisfactory in both procedures but it was slightly higher in the D-SFE method, owing probably to a reduction in the number of steps during the sample handling. The TBT recovery was also higher using the D-SFE procedure.

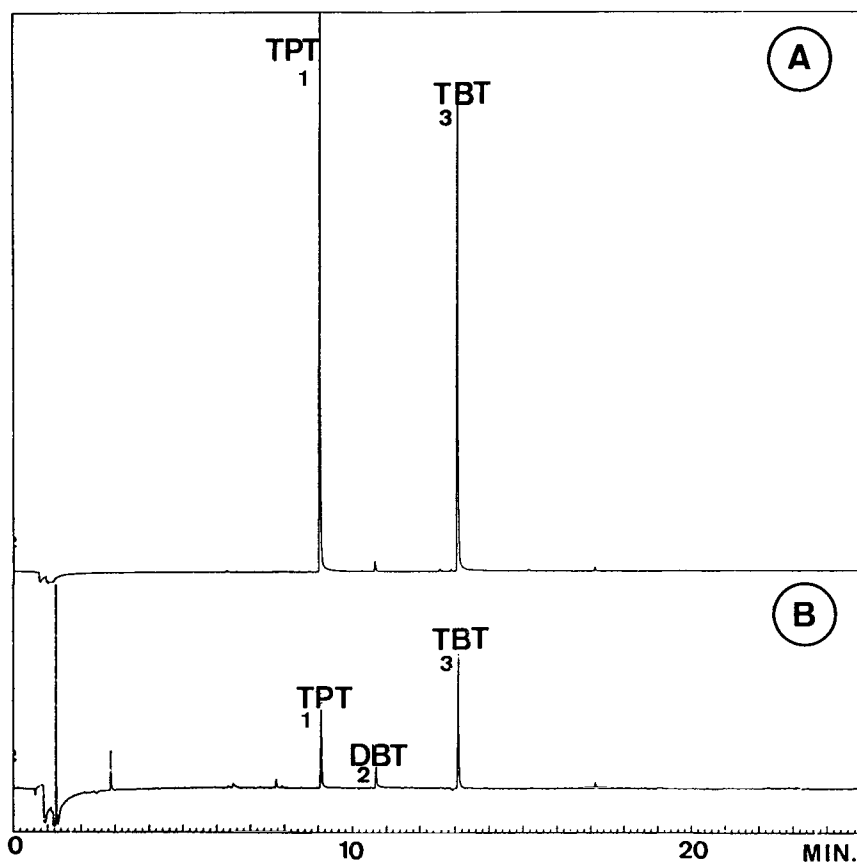


Figure 7: GC-FPD gas chromatograms of: A) standard compounds and B) PACS-1 extracted by SFE-D method. Compound identification as follows: 1, tripropyltin (I.S.); 2, dibutyltin and 3, tributyltin as ethylated derivatives. Analytical conditions are indicated in Figure 5.

The results of both procedures were within the certified range of the CRM PACS-1 for TBT (Table 7). It should be noted that it was not necessary to correct for recovery with the second method used. Nevertheless, recoveries calculated from the certified value were lower in comparison with the spiked sediments, which could be due to a different binding behaviour of organotins in natural and artificially spiked matrices.

Table 7: Accuracy and precision in the TBT determination in PACS-1 by the SFE procedures.

Extraction	Recoveries \pm S.D.*	R.S.D.	[TBT]** ($\mu\text{g.g}^{-1}$, dry mass)
SFE-D (1)	69.4 \pm 6.4	9.2	0.91 \pm 0.9
D-SFE (2)	78.0 \pm 6.0	7.9	0.98 \pm 0.8
Certified value	-----	-----	1.27 \pm 0.2

* Standard deviation from 6 independent replicates.

** [TBT] corrected for recovery in case of method 1.

DBT recoveries calculated from the certified value were significantly lower (38 %) than the ones obtained for TBT by using the D-SFE procedure. Further research is in progress to explain the different extraction behaviour between both matrices.

In addition, another reference material certified for its TBT content was also used for the method validation. This CRM was actually useful to validate the methods with a matrix containing environmentally relevant levels (around 70 ng.g^{-1} as TBT cation [43]). A characteristic chromatogram obtained for the CRM 462 is shown in Figure 8: three compounds were present in all the samples analysed (compounds 1, 5 and 8). They were characterized by GC-MS in the EI mode as dihexyldisulphide and dihexyltrisulphide, respectively [37]. These compounds were assumed to be formed during the derivatization reaction with hexylmagnesium chloride and sulphur compounds present in the sample. However, these compounds did not interfere in the determination of DBT and TBT and hence it was not necessary to remove them. In fact, only monobutyltin co-eluted with dihexyltrisulphide and since the analytical procedure was not successful for this compound, the analytical separation has not been improved.

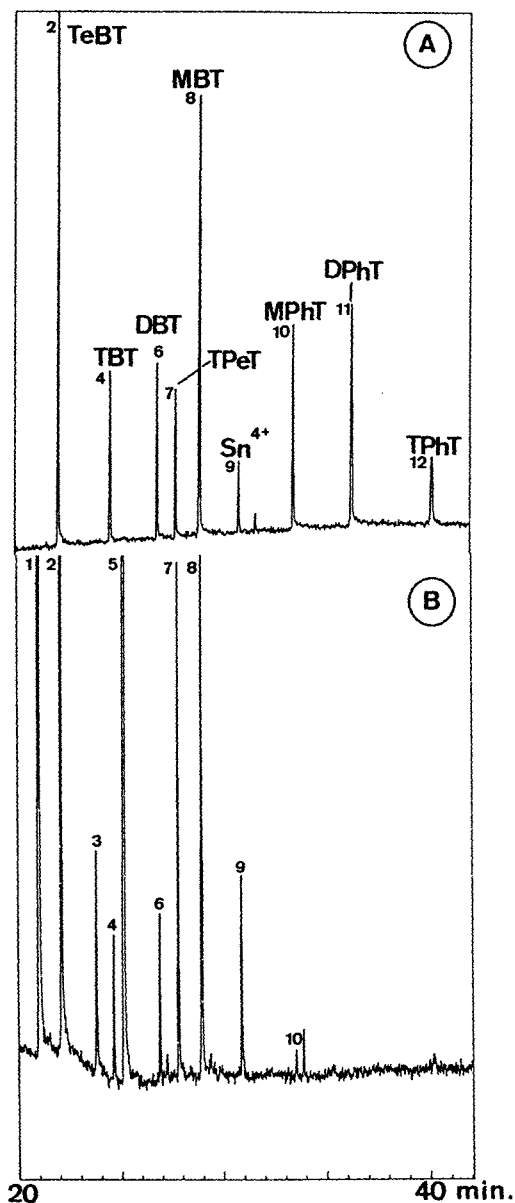


Figure 8: GC-FPD chromatograms of hexylated organotin derivatives. A) calibrants and B) the extract obtained from CRM 462 by the *in situ* sequential derivatization and extraction (D-SFE). Analytical conditions were as follows: fused silica capillary column 30 x 0.25 mm i.d., coated with 0.25 μ m of DB-17. Column temperature was held at 40 $^{\circ}$ C for 3 min and then programmed to 300 $^{\circ}$ C at 8 $^{\circ}$ C.min⁻¹, holding final temperature for 5 min. Helium was the carrier gas at 30 cm.s⁻¹. Compound identification as follows: 1, dihexylsulphide; 2, tetrabutyltin (internal standard); 3, unknown; 4, tributyltin; 5, dihexyldisulphide; 6, dibutyltin; 7, tripentyltin (internal standard); 8, monobutyltin; 11, diphenyltin and 12, triphenyltin.

Table 8: Accuracy and precision for the TBT determination in CRM 462 by the developed SFE procedures

Method	Recovery (%)	S.D.*	R.S.D. (%)	[TBT]** (μg as TBT ⁺)
SFE-D	89	0.016	19.3	0.067
D-SFE	91	0.012	17.4	0.069
Certified value	--	0.014	20.0	0.070

* Standard deviation from 5 independent replicates

** [TBT] determined by the SFE-D procedure was corrected for recovery.

18.3 Application and intercomparison between procedures

18.3.1 Intercomparison between SFE and atmospheric pressure extraction procedure

The developed SFE procedures were compared with an atmospheric pressure extraction (APE) method by analysing the CRMs mentioned above (Figure 9). The APE involved MeOH doped with HCl as extractant agent and the extraction was sequentially performed 3 times. Table 9 summarizes the results showing a remarkable increase of extraction efficiency and precision by the application of the SFE-D procedure. Furthermore, the variance analysis (F-test) exhibited a significant difference between both extraction procedures for PACs-1 and CRM 462.

Table 9: Intercomparison of APE and SFE-D extraction procedures for the TBT determination ($\mu\text{g.g}^{-1}$, dry mass) in reference materials

sample	SFE-D mean value \pm SD*	APE mean value \pm SD*	F-Snedecor	F-confidence 95%
PACS-1	0.91 ± 0.9	0.520 ± 0.03	136.04	5.318
CRM 462	0.083 ± 0.016	0.042 ± 0.017	15.51	5.531

* Standard deviation from 5 independent replicates.

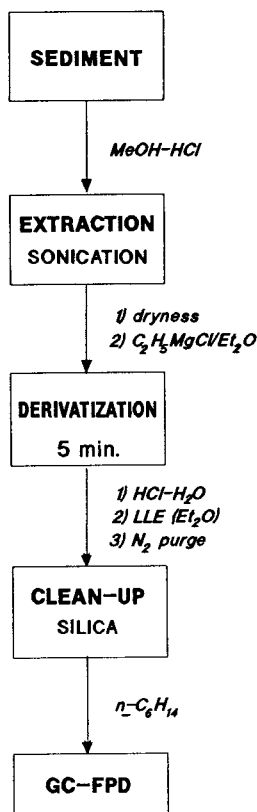


Figure 9: Analytical scheme used in the atmospheric-pressure extraction procedure (APE) using MeOH-HCl.

18.3.2 Application of the SFE procedures for the TBT extraction from real samples

In order to ascertain the validity of the developed D-SFE, a sediment collected from the Masnou marina (Catalonia, Spain) was extracted according to the D-SFE procedure and determined by GC-FPD. Figure 10 shows the organotin distribution with a large predominance of TBT and the quantitative results are listed in Table 10. TPhT and its degradation products were also identified in this sample. In a previous organotin survey conducted in the same area, phenyltin compounds were also identified at high concentrations [23] which is evidence of chronic pollution of these compounds, probably related to their use as biocides in marine antifouling paints.

Table 10: Organotin concentrations obtained by the D-SFE from a NW Mediterranean marina sediment (Masnou).

	TBT	DBT	MPhT	DPhT	TBT
Concentration (ng.g ⁻¹ , dry mass)	994	482	93	20	92
S.D.*	53	78	22	1	14
R.S.D. (%)	5.4	16	24	3	15

* Standard deviation from 3 independent replicates.

The precision of the analytical procedure for the determination of organotin compounds was satisfactory (RSD < 15 %, n=3) except in case of monophenyltin as it was found in the spiked sediment analysed (Table 5). Again, monobutyltin was not possible to be determined owing to a coelution with dihextrisulphide in the analytical conditions used.

18.4 Conclusions

The determination of TBT in sediments by SFE can be achieved using either derivatization with Grignard reagent prior CO₂ or CO₂ modified with MeOH-HCl. Both analytical procedures allowed higher TBT recoveries to be obtained in comparison to a method based on APE with MeOH-HCl sonication. Furthermore, the former procedure allowed the analysis time and the amount of solvent needed to be reduced. Another important advantage of this SFE procedure is a remarkable selectivity, which permits the analysis without any clean-up of the recovered extract since the extraction is performed with the monopolar CO₂. Finally, the SFE procedure developed has been successfully applied to the determination of butyl and phenyltin compounds in real samples. Further research is needed to improve the quantitative results of monobutyl and monophenyltin, which exhibited a poor recovery in all the analytical procedures used.

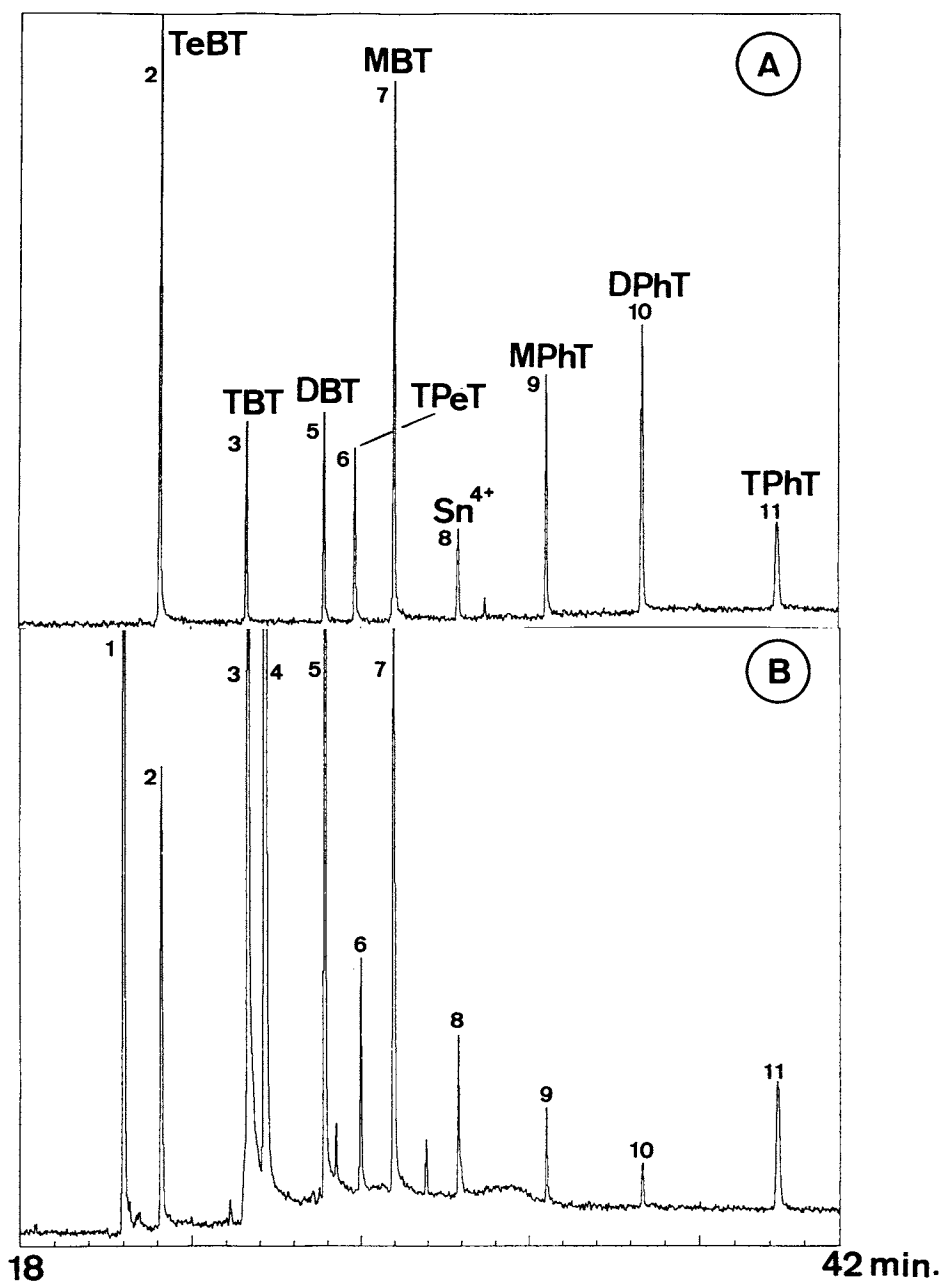


Figure 10: GC-FDP chromatogram of the D-SFE recovered from a marina sediment (Masnou, Catalonia, Spain). Compound identification as follows: 1, dihexylsulphide; 2, tetrabutyltin (internal standard); 3, tributyltin; 4, dihexyldisulphide; 5, dibutyltin; 6, tripentyltin; 7, monobutyltin and dihexyltrisulphide; 8, inorganic tin; 9, monophenyltin; 10, diphenyltin and 11, triphenyltin.

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19.

Hydride generation for speciation analyses using CG/AAS

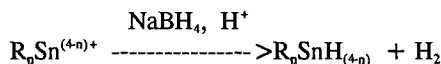
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As mentioned in Chapter 1, most of the techniques used for speciation analysis of environmental samples are based on the coupling of different analytical steps involving extraction, derivatization, separation and detection. Derivatization of organometallic compounds is often required when the separation is performed by gas chromatography but may also be used after liquid chromatographic separation. The major advantage of the derivatization step is the separation of the analytes from the matrix which reduces the occurrence of possible interferences during the subsequent analytical steps and particularly at the detection stage. This procedure also allows pre-concentration of the analytes to be achieved either in the solvent or by cryogenic trapping (cryo-condensation).

Hydride generation is one derivatization procedure which has been extensively used since its introduction by Holak [1] for the determination of total content of arsenic by flame atomic absorption spectrometry. Hydride generation can be performed on-line or off-line and allows both the determination of organometallic compounds and elements with different oxidation states. This procedure may be applied to the determination of a wide variety of chemical species in environmental samples (water, sediment, biological tissues); hydride generation with NaBH_4 can be used for the determination of total contents of elements such as antimony, arsenic, bismuth, germanium, lead, mercury, tin, selenium and tellurium or their species [2], depending upon the exact conditions used.

An example of the hydride generation reaction applied to tin speciation is given below:



with $n = 1, 2, 3$ R is methyl, ethyl or butyl

Hydride generation conditions, *e.g.* concentration of the reductant solution (NaBH_4), pH and types of acids used, must be selected according to the element determined and the quality of the sample [3], as discussed below. Whereas the advantages of this procedure are well recognized, it is often subject to major drawbacks when difficult matrices are to be analyzed. This chapter aims to give an overview of the advantages and limitations of hydride generation when applied to speciation studies.

19.1 Hyphenated techniques using hydride generation

Hyphenated techniques using hydride generation are numerous, *e.g.* gas chromatography (GC) with detection by mass spectrometry (MS), atomic absorption spectrometry (AAS), microwave induced plasma (MIP) or inductively coupled plasma (ICP) atomic emission spectrometry (AES), flame photometry (FPD), or liquid chromatography coupled to AAS, ICPAES or atomic fluorescence spectrometric (AFS) detection [4]. Hydride generation can be performed either on-line (both pre- and post-column separation) or off-line.

Off-line hydride generation with NaBH_4 and simultaneous extraction into dichloromethane has been performed for the determination of organotins by GC/FPD and GC/MS [5]. However, most applications dealt with on-line hydride generation, either pre- or post-column.

On-line hydride generation systems can be divided by their separation mechanism: gas or liquid chromatography. For liquid chromatography, only post-column hydride generation procedures have been described while for gas chromatography numerous applications of pre-column hydride generation have been reported.

Liquid chromatography followed by an in-line photolysis coil coupled with a hydride generator and flame atomic absorption spectrometry (FAAS) is capable of detecting organo-tin and -arsenic species [6-12]. Irradiation with ultraviolet light before hydride generation was used to convert tributyltin and methylated arsenic species into, respectively, inorganic tin and arsenic, from which volatile hydrides could be generated. Recent developments in the HPLC-furnace are based on thermospray nebulization of the HPLC methanolic effluent, pyrolysis of the analyte, gas phase thermochemical hydride generation and cool diffusion flame atomization [13]. This technique allows the determination of organo-arsenic compounds, including arseno-betaine and -choline, and tetramethyl-arsonium salts. The use of post-column hydride generation for speciation of arsenic species by HPLC-ICPAES was also found satisfactory [8,14-16].

Gas chromatography has been used extensively in speciation analysis. For on-line hydride generation in combination with gas chromatographic separation, AAS is the detector most frequently employed. Final detection methods include atomic absorption with an electrothermally heated quartz furnace (QFAAS), flame (FAAS) or graphite furnace (ETAAS). QFAAS is most widely used for speciation analysis for *e.g.* organotins [3], organo-arsenic compounds [17], organo-selenium compounds [3] and antimony [18].

On-column hydride generation allows the direct injection of a solution into the gas chromatograph [19,20]. Derivatization of the extract is performed directly at the top of the column either on solid NaBH_4 pellets introduced at the entrance of the chromatographic column [19] or *via* a packed reactor placed in the injection port of the gas chromatograph [20]. This method has been successfully applied for the determination of Sb(III), Sb(V), As(III), dimethyl-arsenic, methyl- and butyl-tin compounds. However, applications with on-column hydride generation seem to be limited because an extraction step is always required before the injection.

Amongst all the different hyphenated techniques using hydride generation for speciation analysis, the coupling of hydride generation, cryogenic trapping, gas chromatography and atomic absorption spectrometric detection in an electrothermally heated quartz cell is the most widespread; most of the discussions in this chapter will therefore focus on this technique.

19.2 The on-line hydride generation technique in combination with GC/AAS

This technique was developed in 1975 for the determination of methylated forms of selenium in fresh water [21]. It combines four basic steps on-line *viz* (i) derivatization of the analytes, (ii) preconcentration of volatile derivatized analytes by cryogenic trapping, (iii) gas chromatographic separation and (iv) atomic absorption spectrometric detection (Fig. 1). This hyphenated technique has received considerable attention during the last decade owing to its ease of operation, the preconcentration possibilities, the high efficiency of gaseous sample introduction into the detector after cryofocussing and the easiness of automation. Its success lies in the compactness of the system design and the integration of the different analytical steps. The sensitivity of this method allows species of numerous elements to be determined in different kinds of environmental samples.

Several parameters have to be considered in the optimisation of the method and these will be discussed in the following sections.

19.2.1 Reaction vessel

The reaction vessel is usually made of borosilicate glass and the volume ranges from 10 to 500 ml. It must be designed in such a way that the reaction is highly efficient so that the derivatized analyte cannot be trapped within the vessel [22] and that a flow of inert gas may be introduced in the solution for purging and stripping of volatile species.

19.2.2 Reducing conditions

The reducing conditions are particularly critical when dealing with speciation analysis since the chemical forms have to be preserved. The derivatization efficiency strongly depends upon the reducing conditions used for the reaction.

Acidic conditions are necessary in hydride generation using NaBH_4 . The nature and concentration of acid may strongly affect the hydridisation yield; this effect has been observed for the determination of organotin species [27] for which acetic acid (up to 0.2 mol.l⁻¹) gives rise to a higher yield of organotin hydrides than, for example, hydrochloric or nitric acid.

In the hydride generation technique, initial efforts focused on the use of NaBH_4 as reducing agent, in the form of pellets or tablets dropped into the acidic solution in the reaction vessel; it was found, however, that the derivatization yield was only 40-60 % when using pellets in comparison to the yield obtained with NaBH_4 solutions [23].

Typically, the sample solution is purged with a stream of inert carrier gas for a few minutes to remove traces of oxygen. Then an excess of NaBH_4 solution is directly introduced in the reaction vessel, either by syringe injection or with an automated injection device (e.g. using a peristaltic pump).

The concentrations of the NaBH_4 solution used generally range from 10 to 60 g.l^{-1} [24-26]. Higher concentration (up to 100 g.l^{-1}) were used in the case of sediment rich in organic matters [27,28]. NaOH is often added to stabilize the NaBH_4 solution [27]; however, derivatization solutions have to be prepared freshly every day.

In the case of complex matrices rich in organic matter (e.g. biological samples), a significant amount of foam can be produced when NaBH_4 is added to the sample. In these cases, the addition of an antifoaming agent is often necessary [29].



Figure 1: Hyphenated system for speciation analysis (developed at the University of Bordeaux). (a) NaBH_4 storage flask, (b) reaction flask, (c) three-way valve, (d) trapping/chromatographic column, (e) N_2 dewar, (f) quartz furnace atomizer, (g) electrothermal furnace and (h) atomic absorption apparatus.

19.2.3 *Stripping time/cryogenic focusing*

After derivatization, the analyte hydrides are swept out by an inert gas flow to a cold trap which, besides its cryogenic function, also serves as a chromatographic column. This cryofocussing is performed in a small packed chromatographic column (U-tube of 30-45 cm with *ca.* 0.5 cm i.d.) immersed in liquid nitrogen ($-196\text{ }^{\circ}\text{C}$). Stripping can be carried out with helium at a flow rate of *e.g.* 300 ml.l^{-1} in the solution, after a purging time of 15 min. In general, the hydride species are swept out of the trap rapidly, due to the important degassing effect of the H_2 generated, but most authors allow a fifteen minutes stripping time to ensure a complete recovery of the analytes. In some cases, a water trap can be used (*e.g.* simple glass column immersed in a refrigerated liquid); however, there is a risk of condensation of compounds with low boiling points and hence of decrease in sensitivity. The cryogenic trapping procedure concentrates the analytes and therefore drastically improves the sensitivity of the technique [3].

19.2.4 *Gas chromatography*

The bifunctionality of the column has already been mentioned above. The separation of the hydride species is mainly related to their boiling points (sequential desorption upon heating of the column); however, the choice of the chromatographic packing material (*e.g.* chromosorb 60 to 120 mesh coated with non-polar stationary phase such as OV1, OV101, SE50 or SP2100) can provide a better separation and definition of the peaks. Despite the low resolution power of this column (1300 theoretical plates) [30], the combination of separation based both on boiling points and chromatographic properties permits the determination of a large range of organometallic species in the same run. The good repeatability of retention times (*ca.* 3 %) enables the identification of a wide range of species; however, in some cases it is necessary to use a more specific detector such as MS to identify all the compounds (*e.g.* for species with similar boiling points). The determination of methyltin, alkyllead and methyl-mercury species has been performed using a 10-20 % coating of the stationary phase [3,31,32]. Lighter loadings (*e.g.* 3-5 %) are used for methylated arsenic [33], selenium [34] and higher boiling point species like butyltin compounds [16,35]. The separation process of the trapped hydride species starts as soon as the column is removed from liquid nitrogen and is electrically heated (*e.g.* using a nichrome wire); in the case of butyltin compounds, the column has to be heated up to $250\text{ }^{\circ}\text{C}$ (*e.g.* boiling point of tributyltin: $250\text{ }^{\circ}\text{C}$).

Silanisation of the chromatographic column is recommended to achieve a better desorption efficiency and prevent possible interfering effects or on-column recombination between species. The quality of the chromosorb and of the chromatographic phase are important since the material undergoes high variations of temperature.

19.2.5 *Atomic absorption detection*

As mentioned above, flame, electrothermally heated quartz cell or graphite furnace have been used for final detection. In general, FAAS is not sensitive enough for speciation analysis and ETAAS has not been frequently used mainly because of its discontinuous character of atomization.

An efficient and inexpensive atomizer for the final determination of the hydride species is the electrothermal heated silica furnace. The geometrical design of the furnace is critical for obtaining the best sensitivity; a large variety of furnace designs have been tested, from small size quartz tube [3] to 1 m path-length cell [24]. A typical design of quartz tube is presented in Figure 2.

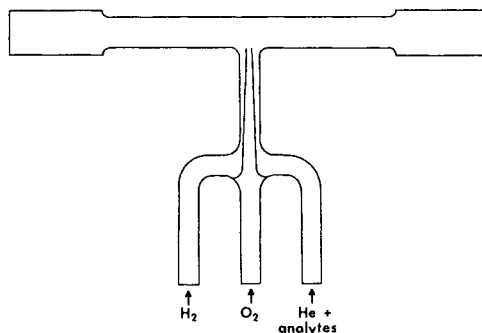
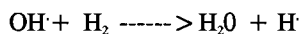
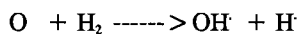
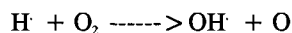


Figure 2: Quartz atomizer design (light path length 20 cm x 0.8 mm i.d.)

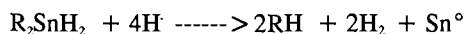
The surface of the quartz cell may be altered by chemical attack due to the presence of matrix components in the tube which might provoke a decrease in sensitivity with time (incomplete atomisation) [3].

An important feature in the atomization step is related to the necessary introduction of additional gases, oxygen and hydrogen, to enhance the atomization efficiency. The presence of H_2 and O_2 will lead to the formation of $H\cdot$ and $OH\cdot$ radicals according to the following equations [36]:



The atomization rate of hydrides is directly controlled by the presence of $H\cdot$ and $OH\cdot$ radicals in the quartz cell. These mechanisms have been described for the atomization of SeH_2 and arsine [36,37]. In optimizing the respective flow rates of O_2 and H_2 , the presence of these $H\cdot$ and $OH\cdot$ radicals leads to an improvement of the atomization efficiency of up to a factor of 1000 [38].

The decomposition of organometallic species proceeds *via* a succession of electrophilic/nucleophilic reactions of the polarized hydrogenated alkylated molecules and either $H\cdot$ and $OH\cdot$ radicals [3]. Atomization of organotin hydrides can be represented according to the following reaction:



The atomic absorption detector can be used without background correction. The use of an EDL is generally preferred on a hollow cathode lamp from a sensitivity point of view.

19.3 Sample pre-treatment

Water samples may be analyzed for butyltins without pre-treatment. However, due to their very high partition coefficient, K_p , water samples should preferably be filtered at $0.45\ \mu\text{m}$, acidified at pH 1-2 and stored at $4\ ^\circ\text{C}$ in the dark to preserve the organotin species [39,40]. The samples can then be analyzed without further pre-treatment.

For arsenic, the storage conditions have to be thoroughly studied since different behaviours may occur from one matrix to another, *e.g.* As(III) was found to be stable in fresh water samples acidified at pH 2 whereas this species is completely oxidized to As(V) within three days in seawater [41]. For seawater, no storage conditions were found to be suitable to stabilize As(III) whereas As(V) is stable both in fresh and sea water at pH 2 [41]. The only procedure to preserve As(III) was by rapid freezing of samples in liquid nitrogen and subsequent storage at $-80\ ^\circ\text{C}$ [33]. Methylated arsenic species are stable over months in acidified water samples or samples stored at $-20\ ^\circ\text{C}$ [42,43]. In general, sediment samples are sieved at $63\ \mu\text{m}$ prior to drying [39]. Biological samples are generally freeze-dried [44]. Solid samples require an extraction step, *e.g.* with acetic acid, prior to the hydride generation. Many studies have been carried out for the optimisation of organotin extraction from sediments. For butyltins, extraction can be performed using acetic acid ($5\ \text{mol.l}^{-1}$) under reflux or a methanol-HCl mixture [40,44-47].

19.4 Applications of hydride generation

Organometallic species have been determined quite often by hydride generation GC/AAS, not only for research applications but also for routine monitoring purposes. This procedure has been successfully applied for the determination of As, Ge, Sb, Se and Sn species [2-4], *e.g.* for monitoring butyltin levels in aqueous samples [21,55,56] and sediment and biota [45]. In addition, hydride generation was used to generate mercury hydride species for the determination of methylmercury hydride, diethylmercury and dimethylmercury species which were found to be sufficiently stable during the analytical process to be determined [57] (the half-life of MeHgH is of ca. 2 h [58]). The existence of methylmercury hydride was confirmed by NMR and mass spectrometry [59]. Hydride generation was used recently to determine dimethylmercury in seawater [60] and in sediment [61]. Attempts to use hydride generation for lead speciation were not successful owing to an insufficient reproducibility, abundant interferences and instability of organolead hydrides [62,63].

Redox speciation is another application for hydride generation GC/AAS. Different oxidation states of metals and metalloids can be determined by pH-selective hydride generation, *e.g.* arsenite can be determined by pH-controlled reduction (pH of ca. 5-6) while arsenate can be determined after acidification with HCl ($2\ \text{mol.l}^{-1}$) [64,65]. Similar determinations have been performed for selenium (IV) and (VI) [66,67], antimony (III) and (V) [18] *etc.* Hydrogen selenide can be generated from selenite in HCl $4\ \text{mol.l}^{-1}$ after addition of NaBH_4 . Selenate is not reduced under these conditions and has to be reduced first to selenite with *e.g.* KBr [34,68]. It is important to control the reducing conditions, *e.g.* iodide should not be used to reduce Se(VI) to Se(IV) since it is a too strong reductant and elemental selenium can be generated which does not react with NaBH_4 to yield SeH_2 [69].

Antimony (III) and (V) can be differentiated by hydride generation since the two species do not form stibine with NaBH_4 under the same conditions. Total Sb is first

determined in KI medium acidified with HCl by stibine generation. Stibine is generated from Sb(III) in citric acid medium, whereas Sb(V) is obtained by the difference of Sb(III) to total antimony [70]. The addition of Sb(V) to Sb(III) to a final ratio of 1:8 does not interfere strongly on Sb(III) determination. Antimony species have been determined in estuarine and sea water by hydride generation of Sb(III) at pH 5 in a sodium acetate/acetic acid buffer [71].

Volatile species in aqueous solution can also be determined with a slightly different approach by hydride generation GC/AAS. Ionic organometallic compounds can form volatile species in the environment after hydrogenation or methylation. Such processes have been shown to occur in the environment, *e.g.* stannane and dimethyltin hydride were detected in natural water and sewage water [72,73] and methyltin and butyltin hydrides were shown to occur in estuarine water [74]. A fraction of these species is present, however, in a dissolved form in water and can be determined by enhancing the liquid/gas exchanges in the hydride generation set up by only stripping off the volatile analytes, without NaBH₄ derivatization. This procedure has also been successfully used for the determination of volatile selenium species [3].

19.5 Interferences in hydride generation

Speciation analysis involving hydride generation can be affected by interferences during the analytical process. Interferences may occur at each step of the analytical determination. In general, the transition elements and the hydride forming elements are strong interferents which potentially affect the final determination of the analytes. Interferences in hydride generation have been observed for total metal determinations as well as in speciation analysis. However, their mechanisms have mostly been studied for total determinations. Interferences can be divided into two groups: (i) spectral and (ii) chemical interferences.

19.5.1 Spectral interferences

Spectral interferences may arise from the overlap of spectral lines of molecular bands; they occur from volatile matrix compounds which are able to absorb at the analyte wavelength and lead to non specific absorption peaks. In the case of hydride generation, spectral interferences are limited since the analytes are separated from the matrix which remain in solution while the analytes are stripped away.

19.5.2 Chemical interferences

Chemical interferences may occur either (i) during hydride formation in the solution or (ii) in the gaseous phase [75].

During hydride formation in the solution the so-called "liquid phase interferences" can partly or completely inhibit hydride generation by competitive reaction with the reducing agent [75]. Another cause of inhibition is the possible co-precipitation of the analyte or the formation of non-soluble compounds [76]. The formation of non-soluble finely dispersed metal is capable of capturing analyte hydrides [77]. In addition, the reaction of NaBH₄ with interferents could lead to the formation of highly reactive metal borides which could also decompose the analyte hydrides [78-80].

Gas phase interferences can occur when different hydride species are transported to the atomizer at the same time. They arise from the presence of volatile interferents produced simultaneously during the NaBH₄ addition [81]. Atomization efficiency depends on the

effectiveness of the collision between radicals and the analyte hydrides. Due to a shortage of radicals, signals can be affected seriously [82]. The use of a quartz tube atomizer generally provides a better sensitivity for the detection of hydrides than flame atomization [83]. However, some interferences may occur owing to the presence of other hydride forming elements in the quartz cell. In some cases, the surface of the quartz tube may deteriorate rapidly, leading to an important decrease in sensitivity (decrease of atomization efficiency). The devitrification of the quartz tube, its state and quality, the presence of traces of vapour of organic solvent, the conditioning of the material are factors which can directly control the yield of the hydride atomization [3]. However, gas phase interferences occasionally occur in the hydride generation procedure but are rather limited when a pre-concentration/separation step is used.

Numerous procedures have been used to decrease interferences in hydride generation. The reduction of interferences can be performed by the addition of a buffer ion which is more easily reduced than interferents, delaying or preventing their reduction and precipitation [84]. An increase of the acidity of the medium [85] probably increases the solubility of the reduced interferents and/or the species formed between interferents and analytes, reducing interferences from transition metals.

Approaches to minimize, or eliminate interferences in hydride generation have been applied more or less successfully. Some methods have been proposed:

- addition of masking agents like EDTA [86-88], potassium iodide [69,89], L-cystine and L-cysteine [96-100] and ascorbic acid [70,89];
- increase of the solution acidity and/or the concentration of the reducing agent [85];
- separation techniques like *e.g.* co-precipitation [71] or application of chelating resins [89].

Howard and Arbab-Zavar [89] have studied different possibilities to suppress interferences in the determination of arsenic species by HG-CT-GC/AAS: dithizone extraction, the use of chelating ion exchange resin chelex 100 and the addition of EDTA as masking agent. The dithizone extraction procedure was the least efficient. The use of EDTA was most recommended to prevent interferences on arsenic speciation using hydride generation.

19.5.2.1 Interferences in organometallic speciation

Interferences in organometallic speciation using hydride generation have mostly been studied with regard to organotin compounds. Inhibitions in organotin speciation were first reported in the presence of diesel fuel in seawater [92]. These interferences have also been observed for the determination of TBT in sediment containing high contents of gasoline [91]. However, these interferences could be overcome by increasing the amount and concentration of NaBH_4 ; particularly, an increased concentration (80 mg.l^{-1}) and volume of reagent (up to 10 ml) were found necessary to reach a plateau corresponding to an assumed complete yield of TBT hydrides. Sulphur compounds and pigments were also suspected to interfere strongly in organotin determinations [93].

Recently, an extensive study has been carried out to investigate the interference effects and mechanisms of various inorganic and organic substances on hydride generation for the determination of organotin species [94,95]. Interference effects were studied on the basis of synthetic solutions containing organotin mixtures (monomethyltin, mono-, di- and tri-butyltin) at concentrations representative of environmental levels, *i.e.* in the range of 5 ng as Sn in 50 ml solution (100 ng.l^{-1}). The possible interferents investigated were

organic pollutants (e.g. PCBs, pesticides, n-alkanes), methanol, humic substances, EDTA and several inorganic compounds. Organic pollutants did not generate serious signal depression even at concentrations simulating extremely polluted areas. The addition of humic substances affected the reproducibility of the determinations rather than the sensitivity which was assumed to be due to the influence of foam production during the stripping step. EDTA was shown to inhibit the determination of monoalkyltins (MMT and MBT) while DBT and TBT were not significantly affected, which was supposed to be due to a more efficient complexation of EDTA with MMT and MBT in comparison to the other compounds. Inorganic compounds were found to suppress the analyte signals more severely. A mixture of 14 elements (Al, As, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn) was added to the analytes (5 ng each) in increasing quantities (from 0.01 μg to 100 μg of each element) to test the multi-element interference effects. The recovery for MMT was slightly affected by the presence of inorganic compounds, even at the highest level of interferents, whereas interferences on butyltin determinations were observed at 1 μg levels of each element; the DBT and TBT determinations were totally inhibited for 100 μg of each element while the inhibition of MBT was about 65%. Interferences were supposed to be related to the severe reduction conditions associated to the NaBH_4 reaction and the subsequent attack of the Sn-H bonds by inorganic species. In the gaseous phase, interferences may occur either during the separation or atomisation steps. It is more likely that these interferences arise in the quartz cell during atomisation because no memory effects were reported on the column, no compound was formed and all the retention times remained stable. In the quartz cell, interferences were thought to be due to the presence of volatile interferents (probably hydrides) generated by the NaBH_4 addition; as these compounds are in large excess, they may decompose thermally and deposit on the surface of the cell due to the deficiency of H radicals leading to an overall decay in the atomisation rate of organotin hydrides [94].

Recently, it was found that when cysteine is added to samples, As(III), As(V), MMA and DMA give the same response under the same optimum acid concentration [96]. It was proposed that arsenate, MMA and DMA (all in the As(V) state) are reduced to As(III) as organo-sulphur-arsenic(III) compounds through the reaction between the arsenic species and the thiol group.

19.5.2.2 Interferences during redox speciation

The differentiation between oxidation states of elements can be achieved by hydride generation since the reaction of each oxidation state is pH dependent. However, Andreae and Byrd [90] have reported that it was not possible to separate Sn(II) and Sn(IV) using hydride generation since both species react with NaBH_4 under the same conditions to yield stannane.

The separation of elements with different oxidation states can be interfered by other elements. Interferences in arsenic speciation have been investigated by Howard and Arbab-Zavar [89] who have studied the interferences of numerous compounds on the speciation of As(III), As(V), monomethylarsenic and dimethylarsenic by hydride generation, cryogenic trapping, selective volatilisation and detection by AAS in a heated quartz tube atomiser. Important signal depression were observed in the presence of Ag(I), Au(III), Cr(VI), Fe(II), Fe(III), Ge(IV), Mo(VI), Sb(III), Sb(V), Sn(II), Mn(VII) and nitrite at different concentration levels. However, no effects were observed for Al(III), Bi(III), Ca(II), Cd(II), Co(II), Cu(II), Hg(II), Mn(II), Mg(II), Na(I), Pb(III), Se(IV), Se(VI), V(IV) and Te(IV) up to 10 $\mu\text{g}\cdot\text{ml}^{-1}$. Perchlorate, bromide, iodide, nitrate,

sulphate and cysteine hydrochloride did not interfere at concentrations up to $1000 \mu\text{g}.\text{ml}^{-1}$. In the same study, it was found that the effects of cationic interferents, except Fe(II) and Fe(III), can be overcome by passing the sample solution through a column of Chelex 100 resin. However, since iron is an ubiquitous element, this procedure is limited. Moreover, the resins are able to retain a proportion of arsenic when samples contain iron. Amongst the identified interfering elements, the effects of only those due to the presence of Sb, Au(III) and nitrite could not be masked by the addition of $0.02 \text{ mol}.\text{l}^{-1}$ EDTA. Interferences during the determination of arsenic species in natural water are not significant since the possible interfering elements are usually at trace concentrations. Other studies also mention the interferences from transition state and hydride forming elements [97].

In solution, L-cysteine reduces As(V) to As(III) while As(III) reduction by tetrahydroborate (III) is enhanced by a factor of ca. 2 due to the presence of L-cysteine [97]. In the same study, it was shown that Pd, Pt, Au, Ag, Ni, Co, Cu and Fe in the range 0.2 to $200 \text{ mg}.\text{l}^{-1}$ interfered with the hydride generation process. The interferences in the hydride generation also resulted in a memory effect, probably due to a residue of finely divided metal particles precipitated on the generator. The addition of $5 \text{ mg}.\text{l}^{-1}$ of L-cysteine increased the interference-free level by 1-3 orders of magnitude while there was no observed memory effect. Hydride forming elements such as Bi, Ge, Sb, and Sn with concentrations up to $20 \text{ mg}.\text{l}^{-1}$ had no influence on arsine generation. The addition of L-cysteine was also found to be very effective in the determination of Sb, Ge and Sn in effectively reducing interferences [98-101].

In Se determination, the use of cyanoborohydride leads to a signal improvement, while the Se signal in natural waters can be completely suppressed by the presence of nitrite [67]. Organic or sulphide-rich matrices may also partly react and compete non-quantitatively during hydride generation [102]. Nitric acid and nitrite interfere by the formation of volatile nitrogen oxide species that cause gas-phase interferences [78,103] which can be overcome by the addition of sulphanilamide or sulphamic acid.

Antimony and selenium interferences in hydride generation were investigated for arsenic speciation [85]. Reaction of these two elements with α -hydroxyacids in the presence of KI allowed these interferences to be limited to a considerable extent.

As said before, very few systematic studies have been carried out on interference effects in organometallic speciation. Hence, much needs to be done to understand possible interference patterns in hydride generation and find possible ways to reduce interferences.

19.6 Other limitations

Due to use of low efficiency columns, separation problems can occur in the identification of compounds with very similar boiling points, *e.g.* monophenyltin hydride and dimethyl-butyltin hydride have the same retention time on an apolar packed GC column [104]. The use of capillary GC is hence necessary for the species identification.

Some organometallic or organometalloid species are not accessible to direct hydride generation which limits its application, *e.g.* triphenyltin and organic arsenic species. A way to solve this problem was to use UV irradiation to break the molecules of arsono-betaine and arsono-choline to render them accessible to hydride generation [65,105]. However, this UV irradiation must be carried out after the separation step; generally this post-column manipulation is only applied after liquid chromatographic separation.

19.7 Conclusions

It is clear that hydride generation is a procedure which is prone to interferences and, therefore, particular precautions should be undertaken to verify the yield of the reaction. As said in Chapter 1, one of the best procedures to check the analytical performance is to use certified reference materials containing incurred chemical species. Hydride generation-based methodologies were actually tested against other methods in interlaboratory studies organized by the BCR. In the case of tin speciation, hydride generation was successfully applied for the determination of butyltin compounds in solution [106] but it failed in the analysis of a complicated sediment matrix (with high contents of inorganic and organic interferents) containing low levels of TBT [107]. This technique was, however, found to be in good agreement with other methods in the certification of DBT and TBT in a coastal sediment [108]. These results suggest that hydride generation should be used with considerable precaution, particularly for sediment analysis. For water, this method seems to be less prone to interferences and can then be used successfully. However, the difficulty of the verification of hydridisation yield and the risks of uncontrolled interference effects makes it a technique not recommended for routine analysis. The use of an internal standard should be required to control the yield of hydride generation which is still yet not common. For tin speciation, tetramethyltin, triethyltinbromide and dimethylbutyltinbromide have been used [17,26,33,38] but much remains to be done for validating this procedure for the determination of chemical species.

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20.

Single and sequential extraction schemes for trace metal speciation in soil and sediment

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The need for trace metal speciation in soils was recognised and its practice well established in agricultural laboratories long before the term "speciation" entered the literature of analytical or agricultural chemistry. The "species" concerned, and determined, in these early and continuing studies are the plant-available "species" involved in plant and animal nutritional deficiency disease and in plant and animal toxicity. The common perception of speciation as the study of the actual compounds in which elements occur in a material is a practicable one for materials that are solutions, as, for example, natural waters. This conception is too restrictive for general application. In most solid samples, including soils and sediments, the determination of chemical species is in most cases a difficult challenge and suitable means to characterize the actual chemical combination in soils and sediments in a broader view is necessary. In practice, for trace metals a more general definition of speciation is required to encompass the requirements of soil and sediment science, among others. Such a definition has been given [1] and is summarised as: Speciation is the identification and determination (or the description) of the *defined* species of an element that occur in a material. The species can be defined:

- 1- by their *function*, *e.g.* as "plant-available" or "exchangeable" forms,
- 2- by the *operation* designed to isolate and determine them *e.g.* as the species isolated in the soil solution obtained by centrifugation or displacement or the moderately reducible species isolated by a particular reagent and,
- 3- as a particular compound or oxidation state of an element *e.g.* as tributyltin or Cr^{3+} .

This chapter will focus on the speciation in soils and sediments using single and sequential extraction schemes. Although the defined "species" should preferably be referred to as "extractable forms", the term speciation will be used throughout the text for facility.

20.1 General aspects of single and sequential extraction

20.1.1 Functionally defined speciation in soils

The speciation traditionally carried out in soil laboratories for the identification of potential soil deficiency or toxicity status by soil analysis *i.e.* for prediction of the likely effects on the food chain, would be categorised as speciation, functionally defined, in which the function is bioavailability. This functionally defined speciation is determined by the analysis of chemical extracts of soils by reagents, often empirically derived, that extract element concentrations which correlate with crop plant uptake or content of that element, or with plant growth, yield or health. The need for a knowledge of element speciation in this context is based on the fact that total soil content of an element is often a poor indicator of its bioavailability.

Most of the early studies of soil speciation have been concerned with deficiency problems in plants or animals and successful extraction procedures have been validated by field experimental correlation between plant response and extract content over several seasons. This aspect of speciation methodology validation will not be discussed in depth in this chapter but soil extractants used to assess bioavailable element contents are summarised in Table 1. Although valid extraction methods for assessment of soil toxicity status for elements such as molybdenum (induced copper deficiency in ruminants) and nickel (plant toxicity) have been evolved, extraction methods for heavy metal toxicants are less well established.

Table 1 Single-step extraction reagents for bioavailable heavy metal species in soils

Extractant	Elements	References
Water	Cd, Cu, Zn	[4-6]
Sodium nitrate (0.1 mol.l ⁻¹)	Cd, Pb	[7]
Ammonium nitrate (1 mol.l ⁻¹)	Cd, Pb	[8]
Calcium chloride (0.05-0.1 mol.l ⁻¹)	Cd, Cu, Mn, Ni, Pb	[9-11]
Ammonium acetate (1 mol.l ⁻¹ , pH 7)	Mo, Ni, Pb, Zn	[13-16]
Ammonium acetate/EDTA (0.5 mol.l ⁻¹ :0.02 mol.l ⁻¹)	Cu, Fe, Mn, Zn	[5,9,17,18]
EDTA* (0.05 mol.l ⁻¹ pH7)	Cd, Cu, Ni, Pb, Zn, Mo	[19-21]
DTPA* (0.005 mol.l ⁻¹ DTPA + 0.1 mol.l ⁻¹ TO* + 0.01 mol.l ⁻¹ CaCl ₂)	Cd, Cu, Fe, Mn, Ni, Zn	[4-6,18,20,22-27]
2.5 % HOAc	Cd, Co, Cr, Cu, Pb, Ni, Zn	[13,19,28-32]

* EDTA = Ethylenediaminetetraacetic acid; * DTPA = Diethylenetriaminepentaacetic acid;

* TO = Triethanolamine

20.1.2 Operationally defined speciation

Speciation by the use of different extractants can be used not only for this assessment of bioavailability but also to isolate and determine elements bound or associated with different soil fractions or phases. Single extractants designed to isolate a particular species are seldom sufficiently specific for that purpose and will in general extract other species to some extent. Such extractants are best regarded as examples of operationally defined speciation as illustrated in Table 2.

An improvement in the specificity of extraction can be achieved by combining single extractants into a sequential extraction scheme in which the residue from one extraction is extracted by the next extractant in the sequence. Such a procedure delimits the access of each reagent more closely to one species or one soil phase.

Table 2 Extracting reagents or procedures and the soil/sediment phase (nominally) isolated

Phase extracted or isolated	Reagent or method of isolation	Reference
Water-soluble, Soil solution Sediment pore water	Water Centrifugation Displacement Dialysis	[33] [34] [35,36] [37]
Exchangeable	1 mol.l ⁻¹ MgCl ₂ 1 mol.l ⁻¹ NH ₄ OAc 0.05 mol.l ⁻¹ CaCl ₂ 1 mol.l ⁻¹ KNO ₃	[38] [38] [38,39] [40]
Organically bound	0.1 mol.l ⁻¹ Na ₄ P ₂ O ₇ 0.7 mol.l ⁻¹ NaOCl 0.05 mol.l ⁻¹ EDTA H ₂ O ₂ /HNO ₃ /NaOAc	[40] [41] [42] [33,43]
Carbonate	HOAc NaOAc pH5 EDTA	[33] [33,34] [39,45]
Mn oxide bound	0.1 mol.l ⁻¹ NH ₂ OH.HCl	[46]
Fe oxide bound	Dithionite/citrate	[47]
Mineral lattice	HF	[48]

20.1.3 Metal speciation in sediments

Trace metal speciation in sediments has its origins in the need for assessing the form of the heavy metal, metalloid and other toxicants in polluted river and estuarine sediments. The mobility, transformation and potential bioavailability of these toxic elements to the aquatic biota and hence their introduction into the food chain depends on their chemical form. Toxicity of an element is also strongly dependent on its chemical form.

The concentration of an element in the aqueous phase associated with a sediment is seldom controlled by the formation of well defined, poorly soluble compounds of the element but is dependent on interaction of the dissolved species with solid sediment and particulate phases by adsorption or coprecipitation [2]. Thus in the water/sediment compartment speciation is largely concerned with the identification and quantification of the different sediment phases with which heavy metals are associated. Furthermore the use of dredged sediment as landfill presents these same problems in a soil context. The primary interest in sediment speciation is not, in the first instance, so closely related to predicting a crop response. In consequence, the emphasis on understanding the physico-chemical processes of fixation, mobilisation and transformation has made the sequential extraction approach the most favoured one. In contrast, in soil studies, the application of sequential extraction methods has been more limited, although its importance in elucidating physico-chemical relationships and in understanding the fixation and mobilisation of trace metals is considerable.

The development of both single and sequential extraction methods has occurred in different laboratories and in different countries and partly in empirical response to local needs. In consequence the experimental results obtained can seldom be compared, either because totally different methodologies have been employed or, despite the use of nominally the same procedure, significant differences in detail invalidate quantitative comparison. This problem can only be resolved by the common use in different laboratories of the same agreed extraction procedure coupled with a rigorous and detailed protocol for carrying it out. While some information may not be accessible with this approach, the advantages of harmonization of methods and conclusions will, in many circumstances, more than justify the limitations. Such an approach has been adopted by the BCR and considerable progress has been made in implementing this policy [3].

20.2 Current methodologies

20.2.1 *Single-step extraction*

The large range of extractants used in single-step procedures for assessing bioavailable species in soils, and to some extent also in sediments and sewage sludges is illustrated in Table 1. This is not an exhaustive list but illustrates the wide variety of extractants used for bioavailability testing and prediction of trace element deficiency or toxicity in crops or animals. The choice of extractant will depend, not only on the element in question but on the weather, the soil type, the crop or animal species and the continuity of the laboratory records. It is obvious that comparison of the results obtained by these different procedures is not easily made.

Soil extraction by chemical reagents, including many of those listed in Table 4.9.1, is also widely used for a more fundamental study of the chemical, physical and microbiological processes that govern the deposition, the mobilization and transport of trace metals and metalloids in soils. Extraction is, in this role, used to isolate the trace elements associated or bound in a particular soil phase, fraction or component. Extractants used in this way are, however, often less phase-specific than is intended or desired and may dissolve elements from other soil phases. Although extractants may be designated as acting on a particular soil phase, their role is more precisely defined by the nature of the reagent, or the procedure used. Extractants or

procedures used with these objectives are thus being employed for speciation as operationally defined. Some examples of extracting reagents and fractionation procedures together with the phase or fraction isolated are given in Table 2.

20.2.2 Sequential extraction procedures

Sequential extraction schemes are many and varied. They range from the early 5-step method of McLaren and Crawford [48] to the 9-step procedure of Miller *et al.* [40], both for soils. Other soil procedures include references [39] and [49]. Procedures designed for sediments include the pioneering work of Presley *et al.* [33] and of Tessier *et al.* [43]. The latter has been one of the most popular schemes and has formed the basis for several modifications, notably that of Salomons and Förstner [50] and including references [51] and [52]. Tabular comparisons of several sequential extraction schemes are presented by Pickering [53] and differences in schemes are discussed in reference [3]. The great variety of reagents and the phases intended to be isolated by them is illustrated for four procedures in Table 3.

20.3 Justification and validation of speciation by extraction

20.3.1 Validation of the relevance and specificity of extracting reagents

In the case of single-step extraction procedures for functionally defined speciation, *i.e.* largely for the determination of bio-available species in soils, validation depends on long-term field experiments to establish correlations between soil extract concentrations and crop plant uptake, element content or growth. This is a principal topic of trace element research in agriculture and is not dealt with here in detail. Some of the literature is noted in Table 1 and other useful references include [3, 54-57]. A tabular comparison of eight sequential extraction procedures for soils is given by Lake *et al.* [58].

The justification for the use of sequential extraction procedures lies in their ability to extract metal species from particular soil or sediment phases. It is generally recognised that most extracting schemes are less than perfect, *i.e.* few extractants can be relied on to release elements solely from a particular phase. In addition redistribution between phases can occur during the sequential procedure [59,60]. Despite these limitations they are often specific enough for useful diagnostic or managerial decisions to be made on the basis of their evidence. The chemistry and specificity of the different reagents used in extraction schemes is extensively discussed in references [3,55,57,61-63] and is again not the main topic of this chapter.

Table 3 Some examples of sequential extraction schemes illustrating the great variety of reagents and phases (nominally) extracted

Ref. [40] (Müller et al.)		Ref. [49] (Shuman)	
Extractant	Metal Phase	Extractant	Metal Phase
H ₂ O	Soluble		
1 mol.l ⁻¹ KNO ₃	Exchangeable	1 mol.l ⁻¹ MgNO ₃	Exchangeable
0.05 mol.l ⁻¹ NH ₄ F	Adsorbed		
0.1 mol.l ⁻¹ Na ₄ P ₂ O ₇	Organic	0.7 mol.l ⁻¹ NaOCl	Organic
0.01 mol.l ⁻¹ NH ₂ OH.HCl	Mn Oxide		
Citrate/dithionite/ bicarbonate	Fe Oxide	0.2 mol.l ⁻¹ NH ₄ Ox, pH3	Amorphous Fe oxides
		0.2 mol.l ⁻¹ NH ₄ Ox + 0.1 mol.l ⁻¹ ascorbic acid	Crystalline Fe oxides
1 mol.l ⁻¹ HNO ₃	Precipitated		
Conc. HNO ₃	Residual	0.11 mol.l ⁻¹ NH ₄ P ₂ O ₇ ·10H ₂ O	Sand, Silt, Clay
Ref. [43] (Tessier et al.)		Ref. [50] (Salomons and Förstner)	
1 mol.l ⁻¹ MgCl ₂	Exchangeable	1 mol.l ⁻¹ NH ₄ OAc	Exchangeable
1 mol.l ⁻¹ NaOAc/HOAc pH5	Carbonate	1 mol.l ⁻¹ NaOAc/HOAc pH5	Carbonate
0.04 mol.l ⁻¹ NH ₂ OH.HCl/25 % HOAc	Fe/Mn Oxides	0.1 mol.l ⁻¹ NH ₂ OH.HCl/0.01 mol.l ⁻¹ HNO ₃	Easily reducible Mn Oxide/Amorphous Fe Oxides
		0.2 mol.l ⁻¹ NH ₄ Ox pH3	Moderately reducible Amorphous Fe Oxides
30 % H ₂ O ₂ /HNO ₃ pH2 then 3.2 mol.l ⁻¹ NH ₄ Ac/20 % HNO ₃	Organic + Sulfide	30 % H ₂ O ₂ /HNO ₃ pH 2 then 3.2 mol.l ⁻¹ NH ₄ Ac/20 % HNO ₃	Organic + sulfide
Conc HF/HClO ₄	Residual	HF+HClO ₄ (5:1)	Residual

20.3.2 Objective

It is not the concern of this chapter to discuss the virtues or defects of the multitude of existing extraction schemes for speciation in soils and sediments but rather to examine to what extent it is possible to devise simple extraction procedures that:

- (a) provide sufficient information to make diagnostic or managerial decisions on deficient soils or polluted soils and sediments,
- (b) can be accepted as a common reference method by different laboratories in Europe and across the globe and enable valid comparisons to be made in any laboratory

and

- (c) are simple and robust enough to be validated to the extent that reference materials can be prepared and the extractable (species) contents certified, using a prescribed extraction protocol.

Much of the work reported here has been the subject of an intensive study under the auspices of the BCR of the European Commission reported in references [3] and [64-67]. Although single-step and sequential extraction procedures are in wide use for both soils and sediments it soon became evident that, in most laboratories, and in the 30 or more laboratories involved in this development work, single-step methods were usual in soil studies and sequential methods were largely the concern of sediment laboratories. In consequence the following discussion of the problems involved in preparing samples for extraction, in the extraction process, in analysis and in the validation of the procedures developed, is mainly in these terms.

20.4 Development of agreed common extraction schemes

20.4.1 *Sampling soils for analysis and the preparation of bulk soil samples as candidate reference materials for certification of extractable contents*

One of the major hurdles in contemplating the preparation of a reference soil is that the contents to be certified are not the total element contents but the contents extractable by a particular reagent or reagents. This has several consequences. Firstly soil extractions, especially those designed for bioavailability assessment, must be carried out in unground, and conventionally, < 2 mm air-dry soil. The use of fresh, field-moist soils was not considered to be a practical possibility for general use. For representative subsampling of a < 2 mm dry soil the minimum mass required lies between 5 and 20 g as illustrated in Table 4, adapted from references [67] and [68]. If we assume, therefore, that a subsample of 10 g is required for each extraction and analysis, that each bottle of a reference material for issue should provide material for say 10 operations and that a stock of say 1000 bottles is reasonable, then a total of at least 100 kg of the reference soil must be prepared. Furthermore this 100 kg must be homogenised and representatively subsampled into 1000 bottles for issue. This is not the relatively trivial problem that is faced in the preparation of finely ground soils for certification of total contents. Since, in addition, we are concerned with trace concentrations these operations must be carried out without metal contamination.

Table 4 Sample masses for representative soil sampling at different mesh sizes. Adapted from Refs. [67,68].

Mesh opening (mm)	Minimum mass (g)	Optimum mass 4 x Min. wt. (g)
0.16	0.0027	0.011 approx
1.0	0.68	2.5 approx
2.0	5.3	20 approx
4.0	44	170 approx

In a BCR-sponsored trial procedure some 150 kg of air-dried (at $<30^{\circ}\text{C}$), $<2\text{ mm}$, sludge-amended soil was homogenised by gently rolling it back and forth on a polyethylene sheet for three days in a clean greenhouse. The whole sample was coned and quartered and one pair of opposite quadrants collected on a polyethylene sheet. The other pair was set aside and not used further. The collected subsample was again mixed by rolling and the coning and quartering process repeated until 64 subsamples of about 2 kg were obtained. A final coning and quartering was then carried out for each of the 64 subsamples and 20 bottles filled by alternate sampling of opposite quadrants from each of them by means of a nylon spatula. In total 1280 (64×20) bottles each containing some 70 g were thus representatively subsampled from the whole material.

To test whether the sample was homogeneous and that the subsamples were representative, 22 bottles were taken at intervals from the whole series of 1280 (*i.e.* bottles number 35, 85, ...195, 235 ...935, 985) and extracted with 0.05 mol.l^{-1} EDTA and with 0.43 mol.l^{-1} acetic acid. The extracts were analysed for the elements Cd, Cr, Cu, Ni, Pb and Zn by ICPOES with the results for EDTA extraction shown in Table 5. Inspection of this table and also the results of a statistical F-Test show that the techniques employed were able to provide a homogeneous bulk soil sample that could be subsampled in a representative manner. This was true as long as the contents of the analysed solution were sufficiently greater than the method detection limit for the analytical precision to be ignored. For Cr in acetic acid this last proviso was barely met but for the other elements determined in acetic acid extracts the homogeneity and representative subsampling were confirmed. The fact that samples were taken in sequence throughout the subsampling enabled a check to be made that there was no progressive departure from homogeneity during the subsampling process. This method of sequential removal of subsamples for homogeneity testing is therefore to be preferred to a random selection.

Table 5 **Results of homogeneity test**

E1	From analysis of 20 different bottles			From 10 analyses from one bottle		
	Mean (m.l ⁻¹ EDTA extractable)	Std. Dev.	RSD %	Mean (mg.l ⁻¹ EDTA extractable)	Std. Dev.	RSD %
Cd	2.51	0.14	5.7	2.58	0.13	5.2
Cr	5.12	0.36	7.0	5.34	0.45	8.5
Cu	24.81	1.45	5.8	25.4	1.36	5.4
Ni	3.18	0.18	5.7	3.28	0.17	5.2
Pb	28.31	1.50	5.3	29.18	1.49	5.1
Zn	68.49	3.81	5.6	70.06	3.59	5.1

Variance ratio*						
Cd	Cr	Cu	Ni	Pb	Zn	F-Test Expected for 25% of values if no significant difference
1.13	1.12	1.11	1.18	0.63	1.01	1.56

$$*Variance\ ratio = \frac{(Between\ bottle\ Standard\ Deviation)^2}{(Within\ bottle\ Standard\ Deviation)^2}$$

Homogenisation of a 100 kg bulk < 2 mm air-dry soil was also successfully achieved by mixing in an argon-filled drum rotating for 4 weeks in a roll-bed. Subsamples, 10 in number, were then taken by spatula from the centre of the drum into bottles. The drum was again rotated for 2 minutes and a second batch of 10 subsamples taken and so on [69]. The conclusions from the examination of this procedure were the same as from the first method.

20.4.2 Sediment sample preparation

The preparation of bulk sediment samples for analysis or as candidate reference materials is, in some respects, easier than for soils since the usual procedure selects the < 63 µm fraction [67,70-72] by wet or dry sieving. It should be noted, however, that, depending on the objective of the analysis, some method of correction for particle size effects, or normalisation by the use of a "conserved" element such as aluminium as an internal standard [62], may be required. The smaller particle size means that a representative sample can be much smaller than is the case with the < 2 mm soil material and, typically, analytical sample sizes are in the range 0.5 - 2 g. In practice, for many purposes the only practical procedure is the use of air-dried material prepared in a manner similar to those described above for soils.

The use of air dried material for sediment or soil speciation raises the question of the preservation of the material and how to store the sample so that the speciation is also preserved. In the case of anoxic sediments this problem is particularly acute and satisfactory methods may as yet not be readily available. Resort may have to be made for such materials to *in situ* methods of speciation. An experimental validation of the homogenisation, by the rotating drum procedure described above for soils, of some 4 kg of air dried $< 63 \mu\text{m}$ river sediment was successful for subsamples of 0.5 g or greater [73].

20.4.3 Temporal stability of air dried soil extractable contents

Valid methods of speciation by soil extract analysis must depend on the persistence of the element species under study throughout the processes of sample preparation and during storage before analysis. This is particularly important for reference materials certified for extractable element contents if the certificate values are to be valid over a period of a few years at least. It is generally agreed that immediate analysis of the fresh field moist soil is the procedure most likely to avoid changes in speciation [74]. While storage of the moist soil in aerobic conditions has been reluctantly recommended [74], the problems associated with moist soils are many. They are inconveniently bulky, are extremely difficult to homogenise and a representative subsample will be very large. Unless stored at low temperatures, a) microbiological activity will continue and changes in speciation can occur and b) oxidation of organic material can take place, with changes in redox conditions and pH which can, in the case of elements with several oxidation states, such as chromium or manganese, result in changes in extractability [75-78]. For manganese in particular it was concluded that there was no storage procedure that completely prevented changes in extractable contents from occurring nor was freeze-drying able to prevent changes in chromium speciation [74,79,80]. Kersten and Förstner [81] have dramatically illustrated the major changes in the relative proportions of the different fractions of heavy metals extracted from a harbour sediment in a sequential scheme following different pretreatment procedures *viz.*, 1) moist sediment extracted under oxygen free conditions, 2) in oxygenated conditions, 3) after freeze-drying and 4) after oven drying at 60°C . The interpretation of the speciation found by the analysis of dried material must therefore be treated with caution.

In practice, however, there is no realistic alternative to the use of air-dried soils and sediments for reference materials and for routine analytical assessment of trace element speciation. Such materials are easy to store, homogenise and subsample using procedures well established for the measurement of total element contents. It was essential therefore to make a careful assessment of the temporal stability of air-dried soil and sediment trace element extractable contents and this has been done in collaborative studies under the auspices of BCR over the past few years. Evidence exists that, although some extractable contents in dried soil increased compared with fresh moist soil extractable contents [76,77], the EDTA extractable copper contents remained constant, mean RSD 6.7 %, range 4.5-9.4 %, over seven years even with different analysts [82,83]. General experience of extractable procedures in agricultural laboratories over several decades suggests that extractable contents of dried soils remain constant over years, at least for some elements and extracting reagents, although much of the evidence is anecdotal.

Table 6 Test of the temporal stability of extractable soil contents: soils stored at room temperature for 3 years and 1 year. Changes (%) in extractable content of Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn. Adapted from Ref. [3].

After 3 year storage at room temperature								
Extract	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
EDTA	+3.5	-31	+5.0	+0.2	-8.7	+5.2	+6.5	+14
After 1 year storage at room temperature								
EDTA	+19	-2.8	+2.6	-	-0.3	-	-12.8	-3.0
HOAc	+7.4	+36	+20.9	-18.2	+63	+10.2	+1.9	+19.6
CaCl ₂	+118	-	+158	-	+1120	+53	-	+100
NH ₄ OAc c	+7.5	+101	+77	+131	+242	-11.3	-18.7	-26.7

Studies of the temporal stability of extractable contents of soils have been part of the on-going development of reliable procedures that can be used for the preparation of reference materials with certified extractable (species) contents. The results of one such study [3] of extractable contents of soil stored at room temperature for periods of 1 and 3 years are summarised in Table 6. For EDTA extracts it can be concluded that over periods of 1-3 years extractable contents of most of the elements studied were stable within 9 %. Aberrant values for Cr and Zn in the 3-year study were not found in the 1-year study. An aberrant Cd value in the 1-year study was not confirmed in the 3-year results. These exceptional results were probably due to analytical imprecision, contamination or sampling errors and were not attributable to a sample storage effect. For the acetic acid extracts the values were stable within about 20 % over the year with the exception of Cr and Mn. The poorer result for Cu for example reflects the poorer analytical precision obtained at the lower concentrations extracted by HOAc compared with EDTA. For the CaCl₂ and the NH₄OAc extracts no conclusion could be drawn on stability because the element concentrations were in most cases too close to the detection limits of the flame atomic absorption spectrometric method of analysis used. Continuing stability studies of extractable contents of soils stored at -20, 20 and 40 °C showed that, after 1 and 3 months storage, no significant changes in EDTA and in HOAc extractable contents of the elements Cd, Cu, Ni, Pb and Zn occurred, irrespective of the storage temperature. For Cr, however, small but significant changes occurred in the soil stored at 40 °C, with the EDTA extractable content decreasing and the HOAc content increasing. Storage at such high temperatures is not to be recommended.

20.4.4 Temporal stability of air dried sediment extractable contents

A recent study of the stability of extractable trace element contents of the 63 µm fraction of an oxic, air-dried, river sediment [73] showed that, over a period of 8 months storage, the extractable contents of the elements Cd, Cr, Cu, Ni, Pb and Zn

after 1, 2, 4 and 8 months were constant within about 10 % as illustrated in Table 7 for step 3 of a simple 3-step sequential extraction scheme. The 3-step extraction scheme was the one adopted by a group of European experts as a common speciation procedure to facilitate valid interlaboratory comparisons and to be used in the preparation of a BCR reference sediment characterised by certified extractable contents. This sequential extraction scheme is described in detail in section 20.5.3 and Table 8.

Table 7 Stability of the extractable contents of an air-dried sediment: Ratio, R_t , after storage for 1, 2, 4 and 8 months, of extractable contents at time t , (where $t=1, 2, 4$ or 8 months) to the extractable contents at start, t_0 , for step 3 (H_2O_2 at pH2 followed by extraction with $1 \text{ mol.l}^{-1} \text{ NH}_4\text{OAc}$ at pH2) of the 3-step sequential extraction scheme. From Ref. [68].

R_t						
Storage time (months)	Cd	Cr	Cu	Ni	Pb	Zn
1	-	0.97	0.91	0.94	0.95	0.97
2	0.90	0.95	0.97	1.01	0.90	1.05
4	1.05	1.01	1.01	0.94	0.94	1.04
8	0.90	0.96	1.03	1.10	0.92	1.17

20.4.5 Other factors affecting the validity and reliability of extraction procedures for speciation

Of the many aspects of extraction procedures that affect quantitatively the amount of trace element extracted by a particular reagent, perhaps the most important, although seldom explicitly considered, is the type of extraction apparatus used. This includes consideration of the type of mechanical shaker and extraction vessel and the method of separating the extract from the soil residue used. The effect is illustrated for two completely different types of mechanical shaker, *viz.* a cell roller and an end-over-end shaker and filtration as compared with centrifugation in reference [84]. The results showed that, for a 1-hour end-over-end extraction with centrifugation, the 0.05 mol.l^{-1} EDTA extractable contents of Cu, Mn and Zn were 77 %, 61 % and 80 % respectively of the contents found with filtration. These differences were attributed to the fact that extraction continued during the filtration process leading to an effectively longer extraction time, a time that could vary according to the type of sample and its filtration rate. In the cell roller 150 ml polyethylene bottles were positioned on their sides on a roller bed and rotated axially at 2 r.p.m. whereas the end-over-end shaker used Imperial quart (approx 1.1l) glass bottles inverted 60 times per minute. In both cases 15 g soil were extracted with 75 ml of 0.05 mol.l^{-1} EDTA at pH 7. The mean extractable contents of Cu, Mn and Zn were, for the cell roller extraction, respectively 13% greater, 17% lower and 10% greater than those obtained

with the end-over-end shaker. The speed of shaking can also produce a significant variation in extractable contents. Comparing two identical end-over-end shakers and vessels operating at rotation speeds of 28 and 44 r.p.m. it was found that, on average, for 0.43 mol.l⁻¹ acetic acid extraction, the mean extractable heavy metal contents (for 7 elements) were 11 % higher with the faster rotation speed and for 1 mol.l⁻¹ ammonium acetate (pH 7) extracts 20 % higher [3].

Other variables influencing the extractable element content found include the temperature at which the extraction is carried out. For some extracts the increase in the rate of extraction with increase in temperature can amount to a factor of 5 for a temperature change from 30°C to 50°C [85] and this, in principle, can result in an increase in the amount extracted as the temperature of extraction rises. In the collaborative studies of extraction procedures under the auspices of BCR the temperature of extraction was kept constant at 20°C ± 2 °C and under these conditions no statistically significant temperature effect could be distinguished.

It can be concluded that close control of the conditions of extraction are necessary for reproducible and reliable extractable contents of soils or sediments to be determined.

20.4.6 Validation of selective extraction procedures for metal speciation in soils and sediments

The urgent need in the field of metal speciation in soils and sediments by selective extraction is the establishment of common procedures. These must be reproducible, relevant to the biosignificant and bioavailable element contents and simple enough for routine application in different laboratories. Finally, such harmonised procedures allow reference material certified for extractable metal contents (species) to be prepared for use in validating the application in different laboratories of the agreed procedures. Two single-step extraction procedures which have been evolved for soils are firstly an extraction with 0.05 mol.l⁻¹ EDTA and secondly an extraction with 0.43 mol.l⁻¹ acetic acid. The validated BCR sequential extraction scheme is a 3-step procedure outlined in Table 8. These schemes, agreed between over 30 European expert laboratories [65], are now being applied to the preparation of soil and sediment reference materials certified for extractable heavy metal contents. It is hoped that these will be available from BCR in 1994.

Table 8 "BCR" Sequential Extraction Scheme: Extractants and phases (nominally) extracted [3].

Extraction	Extractant	Phase extracted
Step 1	0.11 mol.l ⁻¹ acetic acid	Exchangeable
Step 2	0.1 mol.l ⁻¹ NH ₂ OH.HCl, pH 2	Reducible, Fe, Mn Oxide
Step 3	8.8 mol.l ⁻¹ Hydrogen peroxide Then 1 mol.l ⁻¹ CH ₃ COONH ₄ , pH2	Oxidisable-Organic and sulfide

A recent study [87] of the BCR scheme for the sequential extraction of sediments has been made using mineral substrates equilibrated with heavy metal solutions to simulate a polluted sediment. The results have shown that, like most sequential extraction schemes, the specificity of the extraction stages is less than perfect. A study of the BCR sequential extraction procedure [87] has, however, demonstrated that the repeatability and reproducibility are sufficient for practical application and the sum of the three fractions plus the aqua regia soluble contents of the final residue, equals, within about 10 %, the aqua regia contents of the whole sediment.

The remainder of this chapter will be devoted to the description of these validated procedures which attempt to take account of the various problems discussed here. The detailed protocols that must be followed for valid results to be obtained are also highlighted.

20.5 Protocols for valid extraction procedures for the elements Cd, Cr, Cu, Ni, Pb and Zn [3,64,65,73]

20.5.1 Protocol for single-step extraction by 0.05 mol.l⁻¹ EDTA

All laboratory ware is cleaned by washing in 25 % (V/V) HCl and rinsed with distilled water. For EDTA extraction an additional wash with 0.05 mol.l⁻¹ EDTA solution and a final rinse with distilled water is carried out. Where electrothermal atomic absorption spectrometry is used [85], instead of the flame atomic absorption or inductively coupled plasma optical emission spectrometry used in these studies, the hydrochloric acid wash is replaced by a dilute (4 mol.l⁻¹) nitric acid wash.

a) Extracting reagent: 0.05 mol.l⁻¹ EDTA as the ammonium salt

This is prepared by mixing 146.12 ± 0.05 g EDTA (free acid) with 800 ± 20 ml distilled water and dissolving by stirring in approximately 130 ml of ammonia solution until all the EDTA has dissolved. The filtered solution is adjusted to pH 7 ± 0.05 by the addition of hydrochloric acid or ammonia.

b) Soil sample

A representative soil sample (5-20 g), typically 5 ± 0.05 g, of air-dried (at < 30 °C), < 2 mm soil is weighed into a 250 ml polyethylene (or polypropylene or borosilicate glass) extracting vessel and 50 ml of the 0.05 mol.l⁻¹ EDTA extracting solution added.

c) Extraction

The extracting vessel and contents are immediately shaken in an end-over-end mechanical shaker operating at approximately 30 r.p.m. for 1 hour at a room temperature of 20 ± 2 °C. The extract is immediately filtered through an 18.5 cm Whatman 542 filter paper (or equivalent) that has been previously rinsed with the EDTA extracting solution and allowed to drain. The filtrate is collected in a polyethylene bottle for analysis by atomic absorption or inductively coupled plasma optical emission spectrometry. A blank extract (*i.e.* without soil) is analysed along with each batch of samples.

20.5.2 Protocol for single step extraction by 0.43 mol.l⁻¹ acetic acid**a) Extraction reagent: 0.43 mol.l⁻¹ acetic acid**

This is prepared by adding, in a fume cupboard, 250 ml of redistilled or, for example, Suprapur grade glacial acetic acid to about 5 litres of distilled water and making up to 10 l volume in a polyethylene vessel.

b) Soil sample

As above a representative sample, typically 5 ± 0.05 g of air-dried (at $< 30^\circ\text{C}$), < 2 mm soil is weighed into a 250 ml polyethylene extracting vessel and 200 ml of the 0.43 mol.l⁻¹ extracting solution added.

c) Extraction

The extracting vessel and contents are immediately shaken in an end-over-end mechanical shaker, operating at approximately 30 r.p.m. for 16 hours (overnight) in a room at a temperature of $20 \pm 2^\circ\text{C}$. The extract is immediately filtered through an 18.5 cm Whatman 542 filter paper (or equivalent) into a polyethylene bottle for analysis by atomic absorption or inductively coupled plasma optical emission spectrometry. Extracts are stored at 4°C if not analysed immediately. A blank extract, *i.e.* without soil, is analysed with each batch of samples.

20.5.3 Protocol for BCR 3-step sequential extraction for sediment

All vessels are cleaned by soaking in 4 mol.l⁻¹ nitric acid overnight and rinsing with distilled water. The cleaning process is checked by adding 40 ml of the 0.11 mol.l⁻¹ acetic acid reagent to one vessel and analysing the contents with the extracts from step 1. Reagent blanks for each reagent should also be analysed. Standard solutions for the analyses should be made up in the appropriate reagent matrix.

a) Reagents**i) Reagent A: 0.11 mol.l⁻¹ acetic acid**

This is prepared by diluting 0.43 mol.l⁻¹ acetic acid, prepared as above for single-step extraction, 1 + 3 with distilled water.

ii) Reagent B: 0.1 mol.l⁻¹ hydroxyammonium chloride (hydroxylamine hydrochloride)

This is prepared by dissolving 6.95 g of hydroxyammonium chloride in 0.90 l of distilled water, acidifying to pH 2 with nitric acid and making up to 1 l with distilled water.

iii) Reagent C: 30 mg.g⁻¹ (8.8 mol.l⁻¹) hydrogen peroxide solution

As supplied by manufacturer *i.e.* acid-stabilised to pH 2-3.

iv) Reagent D: 1 mol.l⁻¹ ammonium acetate.

This is prepared by dissolving 77.08 g of analytical grade ammonium acetate in 0.90 l of distilled water, adjusting with nitric acid to pH 2 and making up to 1 l with distilled water.

b) Extraction

STEP 1:

A representative sample of air-dried (at $< 30\text{ }^{\circ}\text{C}$) $< 63\text{ }\mu\text{m}$ sediment is weighed into a 100 ml centrifuge tube and 40 ml of reagent "A", 0.11 mol.l^{-1} acetic acid, is added and the vessel and contents shaken for 16 hours (overnight) in an end-over-end mechanical shaker operating at 30 r.p.m. in a room at $20 \pm 2\text{ }^{\circ}\text{C}$. The supernatant is separated by centrifuging at 1500 G and decanting into a polyethylene bottle. This fraction 1 is analysed immediately or stored at $4\text{ }^{\circ}\text{C}$. The residue is washed by shaking with 20 ml distilled water for 15 minutes, centrifuging and discarding the washings. The residue is retained for step 2.

STEP 2:

40 ml of reagent "B", 0.1 mol.l^{-1} hydroxyammonium chloride, is added to the broken up residue from step 1, above, in the centrifuge tube and again extracted at $20\text{ }^{\circ}\text{C}$ as before for 16 hours (overnight). The supernatant is separated and retained (Fraction 2), as before for analysis. The residue is again washed, the washings separated by centrifugation and discarded. The residue is retained for step 3.

STEP 3:

To the broken up residue, in the centrifuge tube, from step 2, 10 ml of reagent "C", 30 mg.g^{-1} (*i.e.* 8.8 mol.l^{-1}) hydrogen peroxide, is added slowly, (little by little to avoid violent reaction and consequent losses). The vessel is lightly covered so that gases can escape, and the reaction allowed to proceed, at room temperature, for 1 hour. After digesting at $85\text{ }^{\circ}\text{C}$ for a further 1 hour, the cover is removed and the volume reduced to a few (2-3) ml by heating. A second 10 ml aliquot of hydrogen peroxide reagent is added and digestion carried out for 1 hour at $85\text{ }^{\circ}\text{C}$. The volume is again reduced to a few ml. After allowing to cool 50 ml of extracting solution "D", 1 mol.l^{-1} ammonium acetate, is added and extraction carried out by shaking for 16 hours. Fraction 3, is separated for analysis, as before by centrifugation.

20.6 Conclusion

It is hoped that with further experience of such agreed and validated common procedures and with the availability, hopefully soon to be realised under the auspices of BCR, of reference soils and sediments certified for extractable heavy metal contents, that the accurate assessment of soil and sediment pollution can be carried out in any laboratory. Moreover, the need to use common extraction protocols to achieve a worldwide comparability of data would justify that these schemes be adopted as international norms by *e.g.* ISO which is presently under discussion.

The desirable extension of such procedures to neutral salt and other weaker extracting reagents, including calcium chloride, ammonium acetate, sodium nitrate or ammonium nitrate solutions depends on the development of analytical methods, such as electrothermal atomic absorption spectrometry, which is more sensitive than flame atomic absorption and the inductively coupled plasma emission spectrometric methods mainly used in these studies. Such development work is currently in progress.

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21.

Methods for the determination of chlorinated biphenyls in air

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Chlorinated biphenyls (CB) are ubiquitous air contaminants. Their concentration in the lower part of the troposphere, expressed as sum of the detected congeners, is generally in the range of 10-100 pg.m⁻³ in polar, oceanic and continental clean air and in the range of 1-10 ng.m⁻³ in the air of urban and industrial regions.

If compared with other matrices (food, water, soil), air generally plays a negligible role in the direct exposure of man and animals to chlorinated biphenyls, but plays an outstanding role in their transport to the other environmental matrices. The analysis of concentrations, patterns and time trends of chlorinated biphenyls in air helps to characterize their sources and to understand the mechanisms of their distribution. Compared to other matrices (water, soil, biota, waste) the processes of uptake and release of organics in the air compartment can be very fast and profoundly influence the sampling practice as well as the elaboration and interpretation of the analytical results. A basic knowledge of the exchange phenomena within the troposphere and between this and the other environmental compartments is thus a necessary premise for a correct sampling. The mechanisms of the global transport and the fate of organics have been reviewed by Ballschmiter [1,2]. The data on the global air-sea exchange of CB, HCH, HCB and DDT have been reviewed by the GESAMP [3]; recent data are reported from the Atlantic [4,5], the Mediterranean and Arabic Sea, the Pacific, Indian and Southern Oceans [4].

21.1 Airborne chlorinated biphenyls

When measuring airborne chlorinated biphenyls it is important to remember that the air is a multiphase system, constituted of gases, organic and inorganic particles and in some cases hydrometeorites (fog, clouds, rain, snow and ice). The partition and the exchange processes of chlorinated biphenyls in the multiphase air compartment can be very relevant for their analysis, *e.g.* chlorinated biphenyls and other low volatility substances can be adsorbed onto particles (*e.g.* condensation nuclei) included in rain droplets, so accounting for an apparent oversaturation which was unpredicted from the low water solubility of the substances [6].

Although the geographical denomination of air samples usually indicates just the site of collection, the content of the sampled material accounts for a combination of processes that happened elsewhere, often in a range of hundreds or thousands of kilometers and over long time periods. When measuring the concentration of airborne chlorinated biphenyls at the same site, it can be common to observe variabilities of factor 5 to 10 or even 20 [7], according to history of the air masses. In spite of the tremendous development of the sampling techniques, the present practice of air sampling could be compared with that of a photographer taking few pictures of moving subjects with a rudimentary camera.

As it happens for any organic substance, the partition of the chlorinated biphenyls in the phases of the air compartment is regulated by the physicochemical properties of the substance (mainly: vapour pressure, polarity and water solubility) and by those of the partition system (mainly: temperature as well as amount, diameter, total surface area and chemical composition of the aerosols).

Chlorinated biphenyls can be removed from the troposphere:

- a) by chemical (*e.g.* OH radicals) or photochemical degradation;
- b) by moving out into the stratosphere;
- c) by gas-diffusion through the interface of the troposphere with water, soil and vegetation, leading to adsorption or absorption by the lower phase;
- d) by dry deposition, adsorbed onto falling particles;
- e) by wet deposition, *i.e.* dissolved in raindrops or adsorbed onto particles included in the raindrops.

In all cases a congener specific discussion is mandatory.

When scheduling a sampling, precipitation (*i.e.* rainfall) should be taken into account because of its important role in the removal of chlorinated biphenyls via removal of dust from the air [6]. Nevertheless most of the theoretical treatment of airborne organics and most of the sampling practice consider the air basically as a biphasic system, constituted of

gases and solid particles, defined as solid aerosol or TSP (total suspended particles). The common range of concentration of TSP is $1\text{--}10\ \mu\text{g.m}^{-3}$ over the open sea, $10\text{--}50\ \mu\text{g.m}^{-3}$ over the land and $50\text{--}100\ \mu\text{g.m}^{-3}$ over urban areas. According to their diameter, the TSP can be divided into coarse particles ($> 2\text{--}2.5\ \mu\text{m}$), mid-size or accumulation mode particles ($2\text{--}0.08\ \mu\text{m}$) and Aitken-nuclei ($< 0.08\ \mu\text{m}$) [8]. The most important adsorption parameter is ϕ , *i.e.* the aerosol surface per unit volume of air ($\text{cm}^2\ \text{cm}^{-3}$). Airborne particles originate from soil erosion, marine spray, biota and industrial activities. In urban and rural air, total carbon in the particles comprises about 10-20 % of the load [8].

The partition of airborne organics between the gas phase and the particles can be predicted with good approximation with the Junge equation [9], whose parameters consider the vapour pressure of the substance, the adsorptivity of the particles and of the substance, their amount and their specific surface area. In principle, the different chemical composition of the particles could influence the adsorption phenomena, but in practice the investigation of this factor is not simple. The approximation of not considering the chemical differences between particles gives an agreement within a factor 3 between experimental results and predictions from Junge's model [8]. The temperature dependence of the gas/particle equilibrium has been described with the Yamasaky equation [10,11].

These theoretical tools are useful when planning a sample collection. High values of total surface area and adsorptivity of the airborne particles (*e.g.* in urban areas), as well as low temperatures can cause half or more of the pentachlorinated and of the higher chlorinated biphenyls to be bound to particles.

The physicochemical properties of the chlorinated biphenyls cover a very wide range of values, according with their chlorination degree. The most important parameter for the air mobility, the vapour pressure, has a range of four orders of magnitude. During the multiple exchanges between the air and the other environmental compartments, the individual chlorinated biphenyls can in part undergo different distributions and fates. Changes in the chlorinated biphenyls profile in the retained material (in the troposphere) and in the fall-out (rain, fog, hoar-frost, snow) has been observed in urban areas [6,7]. On a global scale, a sort of environmental fractionation selectively influencing the global transport of organic compounds and possibly the CB profile has been postulated [12]; for the CB this hypothesis seems controversially supported by the few available data [2,13].

Almost all the samplings of the airborne chlorinated biphenyls have been performed in the first few meters of the troposphere. Using an aircraft, CB and other semi-volatile organochlorine compounds (SOC) have been sampled over the western US coast and sea at altitudes between 100 and 3000 m [14]. Notwithstanding a high variability from flight to flight, the levels of SOC in the upper troposphere were within a factor two in comparison to ground based measurements. The same aircraft measurements showed that a vertical gradient for No_x , SO_2 and other inorganic compounds but no gradient for the SOC; this indicates a good vertical mixing of the SOC in the troposphere which is in agreement with half-lives in air of the order of months [15].

The sampling of airborne substances can follow three principles: condensation by low temperature, absorption (physical or chemical) in liquids and adsorption onto solid surfaces [16]. The first two techniques are not much suited for trace analysis because their precision is only satisfactory with much greater concentrations; furthermore, the undesired condensation of airborne water can heavily interfere. In most of the sampling of airborne chlorinated biphenyls an air flux is pulled through various types of trapping systems. Time or flux measurements enable the volume of sampled air to be known. In most cases the volume is 500-2000 m³ (high volume sampling), the duration 12 to 24 hours and the flux 50-100 m³.h⁻¹. The sampled material is then extracted from the trapping devices by heating or with solvents, in some cases cleaned up and fractionated and finally analysed with gas or liquid chromatography. Most of the sampling techniques are designed to separately quantify the amount of chlorinated biphenyls in the gas phase and in the particle phase.

21.1.1 Filter and absorbent systems

Nylon nets impregnated with silicon oil [17] were used in the seventies as passive adsorbents and exposed for weeks or months. Now the most commonly used sampling systems are active samplers, usually consisting of a high volume pump, a filter to retain the particles and an adsorbent to retain the substances in the gas phase.

Filters can be made of glass-fibre or of organic materials [18-22]:

21.1.1.1 Glass-fibre filters

Glass-fibre filters are depth-filters consisting of a tridimensional web of fibers with no defined pore diameter and a high capacity; they generally do not react with the sample components nor adsorb them, can be decontaminated at high temperature (*e.g.* 24 hours at 500 °C) and are fragile to manipulate.

21.1.1.2 Organic filters

Organic filters are made of polymers like cellulose acetate, polytetrafluoroethylene (PTFE) or polyamide; they are surface-filters with a defined pore diameter (0.1 - 5 µm), have limited capacity, can react with some sample components or adsorb them, cannot be purified by heating at very high temperature (*e.g.* PTFE can be heated up to 180 °C only) and are less fragile than glass-fibre filters. Prior to use, their purification can require repeated solvent treatments (*e.g.* by Soxhlet).

Adsorbents can be divided into three categories: inorganic, carbon-based and polymers:

21.1.1.3 Inorganic adsorbents

Inorganic adsorbents are aluminium oxide (Al₂O₃), silica gel (mSiO₂-nH₂O), magnesium silicate (84 % SiO₂, 15.5 % MgO, 0.5 % Na₂SO₄, *e.g.* Florisil), alumino-silicates (MeO_x(Al₂O_m)_n(SiO₂)_o(H₂O)_p, *e.g.* molecularsieve) and porous glass. They have generally high adsorptive surface area (hundreds of m².g⁻¹) and can be purified at very high temperature (500-600 °C); most of them are much hygroscopic and the adsorption of high quantities of water can modify the adsorptivity during the sampling.

21.1.1.4 Carbon adsorbents

Carbon adsorbents can be made of carbon (*e.g.* CF-1), active carbon (*e.g.* CF-17, CPP-9, CPP-16, Supersorbon) or graphitized carbon (*e.g.* Carboxpack, Carbosieve, Carbochrom, Carbosil, Carbosphere). They have a very high surface area (up to 1000 m².g⁻¹), a high adsorptivity for planar aromatic molecules and a low chemical reactivity. Disadvantages are possible catalytic effects, mechanical fragility, incomplete or late elution of some analytes (or even in some cases no elution at all).

21.1.1.5 Polymeric adsorbents

Polymeric adsorbents can be: polyurethan foam (*e.g.* PUF), 2,6- diphenyl-p-phenyl oxide (*e.g.* Tenax GC), polystyrol (*e.g.* Chromosorb 106), styrol divinyl benzene (*e.g.* Porapak P, Chromosorb 101 and 102, Amberlite XAD-2, -4 or -7), vinyl pyrrolidon (*e.g.* Porapak N or R), tetrafluoroethylene (*e.g.* Chromosorb T), methacryl esthers (*e.g.* Amberlite XAD-1 or -8) and many other polymers of various compositions.

The most widely used polymers are cross-linked and are obtained by polymerisation of pure monomers (homogeneous polymers) or of mixed monomers (heterogeneous polymers). Because of their wide variety, the chemical and physical properties can be very different and extended over a wide range of values. Some of their most important parameters are: polarity, selectivity, surface area per mass, thermic resistance, ease of desorbing the analytes.

As previously seen, the distribution of the chlorinated biphenyls between the gas phase and the suspended particles results from a delicate reversible equilibrium. This can be sometimes altered by the sampling techniques leading to imprecise and inaccurate results. Gaseous analytes can be trapped onto the particle filter or onto the trapped particles, leading to an over-estimation of the particle adsorbed fraction (blow-on) [11,23-25]. Furthermore, the pressure drop can evaporate some of the water from the particle surface, increasing the adsorption sites for organic compounds [25].

Some of the particle-adsorbed chlorinated biphenyls can also desorb from the particles trapped onto the filter (blow-off) and can be collected by the adsorbent, leading to an over-estimation of the gaseous fraction [11]. This inconvenience could be partially avoided by frequent changes of the particle filter [26].

Another possible effect is the bleed-off of complex mixtures of alkanes and other compounds from the particle-filter and their adsorption by the back-up adsorbent; here they can act as a stationary phase to co-sorb the gaseous organochlorinated compounds (OC) [27]. It was shown that the adsorption of SOC was very low when two adsorbents were used in series, instead of a filter and an adsorbent [27].

A possible inconvenience of the adsorbents is the break-through of a part of the analytes, downstream of the adsorbing material. This can be checked with a second adsorbent unit, mounted downstream of the first unit.

After sampling, particle filters and adsorbents are separately extracted by ultrasonication or by Soxhlet, using semi-polar solvents or mixtures of solvents (*e.g.* CH₂Cl₂ or hexane/acetone 1:1). From some adsorbents (*e.g.* carbotrap) the chlorinated biphenyls can be extracted by thermal desorption (TD) and directly determined by gas chromatography (TD-GC) [28].

Prior to analysis, the eluates can be treated and analyzed in the same way as the extracts of other environmental matrices. Usually, no special clean-up is required. The extracts can be treated with liquid chromatography (LC) or high performance liquid chromatography (HPLC) in order to separate interfering compounds or to fractionate the analytes (e.g. pesticides/CB or ortho/non ortho CB). The final determination can be carried out by HPLC with UV-detection or more often (when high selectivity and sensitivity are required) by high resolution capillary gas chromatography (HRGC) with electron capture detection (ECD) or mass spectrometric detection (MSD).

21.1.2 Other sampling systems

In order to avoid difficulties in the use of particle filters, alternative systems composed of an adsorbent unit and of different particle-trapping units have been developed:

21.1.2.1 Annular denuders

Annular denuders [29-31] consist of an adsorbent-coated multi-tube which should remove the gaseous substances and let the particle flow finally be trapped by a filter connected to an adsorbent. A classical filter-adsorbent sampler is operated in parallel. In a comparative study with other systems, Kaupp [19] defined the denuders as theoretically ideal but not yet reliable, still being affected by some drawbacks such as e.g. incomplete removal of the gaseous analytes, temperature sensitivity and limited flow capacity.

21.1.2.2 Electrostatic precipitators

Electrostatic precipitators trap the particles from an air stream flowing through a tubular electrofilter. The gaseous analytes are collected by a coupled adsorbent unit. In comparison with glass-fibre filters and impactors, Kaupp [19] and Umlauf [18] found this method unreliable because of secondary reactions of chloro-organics and polycyclic aromatic hydrocarbons onto the filter.

21.1.2.3 Low-pressure cascade impactors

Low-pressure cascade impactors trap the particles *via* impact with the surface of a battery (e.g. fivefold) of funnel-shaped obstacles. The gaseous analytes are then collected by an adsorbent unit. Comparing the glass-fibre adsorbent system with the impactor adsorbent system, Kaupp [19] and Umlauf [18] showed that the latter adsorbed higher contents of particle-bound fractions for CB 52, 101, 153, 180 and for DDT, DDE, HCH, HCB, tetra- and pentachlorinated benzenes and pentachloroanisole. The authors suggested that the particles in the impactor were better protected from desorption of SOC (possibly temperature mediated) than those trapped by glass-fibre filters. As a result of this comparison the authors found differences within a factor two and on average 36 %; they concluded that artifacts by the glass-fiber/adsorbent systems are less drastic than discussed in the literature.

21.2 Deposition of chlorinated biphenyls

The study of the amount and composition of the chlorinated biphenyl mixtures deposited from the air gives indirect information on the air burden and on its transfer or exchange processes with water, soil and vegetation. Three matrices have mostly been investigated: falling particles (dry deposition), precipitations, *i.e.* rain, fog, snow, hoar-frost (wet deposition) and above ground vegetation (foliage, mosses, lichens). Furthermore, the analysis of surface water of lakes [32-36] and seas [3,4,13,37-39] has provided information on the air/water exchange of the CB.

21.2.1 Dry depositions

Dry depositions (aerosols) can be investigated by means of various collecting surfaces and devices. Noll [40] and Holsen [41] used deposition plates covered with Mylar strips coated with Apiezon L grease and mounted on a wind vane. In the same study, Holsen [41] used a multistage rotary inertial impactor that allowed coarse particles (6-100 μm) to be collected by rotating four rectangular collectors covered with Mylar strips coated with Apiezon L grease. The results of the two systems were comparable but the rotatory impactor allowed a size discrimination amongst four classes of particles, showing a decrease of the total CB amount with increasing particle size.

Snow samples were also used to evaluate dry deposition: superficial snow collected after 15 days of sunny weather was used to evaluate the deposition per day and square meter [42].

21.2.2 Wet depositions

Wet depositions of chlorinated biphenyls were investigated by collecting rain samples in glass or stainless steel vessels (30 cm diameter) and extracting them three times with methylene chloride [43,44].

21.2.3 Vegetation samples

Vegetation samples (leaves, needles, tree bark, mosses, lichens) were also used as indicators for evaluating the chlorinated biphenyl levels in the air. In most cases the approach has been qualitative [45-50]. Vegetation/air bio-concentration factors (BCF) for CB and other SOC were determined in laboratory and in environmental conditions [48,50-55]. The use of vegetation samples and of BCF to calculate mean air levels of CB showed in some cases a good correlation between measured and calculated values [56]. Air/vegetation exchange of organic chemicals, including chlorinated biphenyls, was thoroughly reviewed by Paterson [57].

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22.

Sample handling and determination of carbamate pesticides and their transformation products in various matrices

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The use of non-persistent carbamate pesticides, which often replace organochlorine and organophosphorus compounds, is increasing because of their broad spectrum of activity, high pesticide effectiveness and generally low mammalian toxicity. Suitable analytical procedures for the determination of these pesticides in a variety of environmental matrices are therefore required. Analysis of the degradation products of the carbamates is also of interest, since their toxicity may be higher than that of the parent compounds.

A large variety of carbamates is currently in use. Their application as an insecticide, fungicide or herbicide is related to the molecular structure. The insecticides have the N-substituted carbamate moiety and, generally, an aromatic ester or an oxime function. The fungicides have either a benzimidazolyl ester, a pyrimidyl ester or a (bis)dithiocarbamate group. The herbicides have an N-alkylthiocarbamate or an N-phenyl carbamate group [1-3]. Figure 1 shows a general representation of these subclasses.

The degradation products are generally oxidized derivatives, e.g. aldicarb sulphone (from aldicarb), or alcohols formed by saponification of the carbamic acid group, e.g. 1-naphthol (from carbaryl). The general degradation pathways of carbamate pesticides were discussed by Schlagbauer and Schlagbauer [4], while several reports deal with fate studies of specific carbamates: aryl N-methylcarbamates [5,6], benomyl [7,9] and oxime N-methylcarbamates [10,14]. The biodegradation of some carbamates in various environmental matrices has also been studied [15].

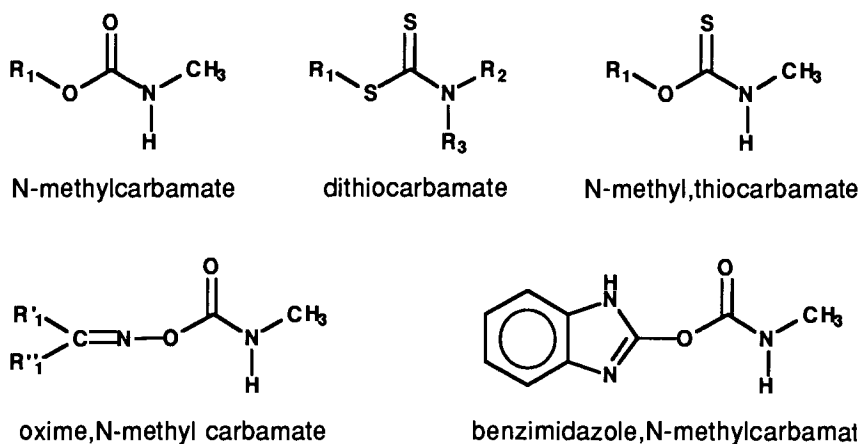


Figure 1 General structures of some subclasses of the carbamates.

Many analytical methods have been developed for the determination of the carbamate pesticides. In addition to the currently most common analytical techniques such as gas chromatography (GC), liquid chromatography (LC), and supercritical fluid chromatography (SFC), other methods, *e.g.* bioassays [16], stopped flow chromatographic techniques [17], thin layer chromatography [18] and colorimetry [19-21] have been employed. This chapter will focus on GC-, LC and SFC based analytical methods.

The rapid growth of the use of carbamate pesticides over the last two decades requires that validated analytical procedures be developed. It should be possible to determine low concentration levels down to *e.g.* 0.1 $\mu\text{g.l}^{-1}$ for drinking water [16]. Furthermore, multiresidue analytical procedures, which comprise the carbamates and their transformation products, are of much current interest, because their use effectively reduces analysis time and expense.

Amongst other, our group, with the support of the Community Bureau of Reference (BCR, Measurements and Testing Programme, Brussels) of the European Commission, has developed validated extraction, clean-up and analytical methods for the determination of carbamate (and some other) pesticides in different environmental matrices. So far, several review papers concerning the use of LC [22-24] and mass spectrometry (MS) [25] have been published.

In the following, procedures for LC, GC and SFC analysis of some subclasses of carbamates (and their degradation products) in various matrices will be discussed. The chapter, divided into three sections, covers: (1) sample preparation from water, plant materials and soil, (2) LC, GC and SFC separations and (3) ultraviolet (UV), fluorescence, electrochemical (ECD) and mass spectrometric (MS) detection in LC.

22.1 Sample preparation

Samples which may contain carbamates should be treated with care, because the compounds are susceptible to degradation due to, *e.g.*, hydrolysis at basic or neutral pH [35], prolonged exposure to light and metabolism. Adjustment of the pH of an aqueous sample, *e.g.* carbofuran, from pH 9.5 to 6.5, keeps the solution stable for one week. Therefore, aqueous samples should generally be acidified and extracted directly after collection. Exposure to light may be minimized by storage in the dark and metabolism may be prevented by performing the extraction directly after sampling. Since the increased polarity of the (bio)degradation products will generally add to the complexity of the problem, extraction (and clean-up) requires careful attention.

Various procedures have been reported for the extraction of carbamates and some of their transformation products from environmental matrices such as surface or ground water [26-43], plants [44-55] and soil [53-57]. The extraction procedures for water samples, biological materials and soil were recently reviewed [58]. The EPA procedure for the determination of N-methylcarbamates in surface water [40] requires neither extraction nor clean-up (500 μ l samples are injected into the analytical system). Extraction by liquid-liquid, solid-liquid, solid phase or supercritical fluid extraction (LLE, SLE, SPE and SFE, respectively) may be applied. In LLE the analytes are partitioned between two immiscible liquid phases, generally water and an organic solvent. In SPE the analytes are first adsorbed from a liquid sample onto a solid sorbent and then selectively desorbed, often after washing of the pre-column. In SLE the analytes are partitioned between the solid sample and a liquid phase, generally an organic solvent and/or water. In SFE the analytes are directly extracted into a supercritical fluid. LLE and SPE are commonly used for water samples, whereas SLE, using *e.g.* soxhlet extractors or sonication, and SFE are mainly applied to foodstuff, soil and sediment samples.

Clean-up is generally required for foodstuff, sediment or soil extracts, using *e.g.* liquid-liquid partitioning or adsorption chromatography. Extracts of aqueous samples hardly ever require further clean-up.

The efficiency of sample preparation, both for extraction and clean-up, is usually tested by recovery experiments, in which samples are spiked with known quantities of the analyte. The recovery itself, as well as its reproducibility, as a mean square root standard deviation (RSD), are the key quality parameters for sample treatment. If the efficiency is high (over 80%) and reproducible (RSD values of a few percent), a correction for analyte losses can safely be carried out. The selection of a sample preparation method is strongly dependent on the recovery and its reproducibility and on the sample type (aqueous, solid, biological), but aspects such as treatment time and consumption of chemicals should also be considered. In the following, sample preparation for aqueous samples, biological material and soil samples (mostly solids) will be discussed separately.

22.1.1 Aqueous samples

A variety of parameters influences the extraction efficiency of LLE and SPE, which are commonly used for the extraction of water samples. Although LLE is still applied, SPE is currently of more interest, mainly because it can be coupled on-line in an analytical procedure. The most important extraction parameters are discussed below.

Various solvents may be used in LLE to obtain a satisfactory partitioning of the analytes between water and an organic phase. Dichloromethane is mainly applied for carbamate extraction, although chloroform, toluene and benzene are also used [58, and references cited therein]. The partitioning can be influenced by the acidity (pH) [35] and ionic strength [29] of the solution, the nature of the sample (*e.g.* presence of particulate material) and the volume ratio of the phases. Moreover, the overall extraction efficiency depends on the number of subsequent extractions. In a typical extraction procedure, one litre of water is acidified and extracted with three portions of the solvent (totalling 100 ml). The extract is subsequently evaporated to dryness and, prior to analysis, redissolved in a small volume of a suitable solvent.

A variety of sorbents may be used for extraction and clean-up with SPE, *e.g.* Amberlite XAD resins, Carboxpack B and bonded silica phases. Many sorbents are commercially available, often prepacked in disposable cartridges. The choice of an appropriate sorbent is of crucial importance for the extraction and, hence, several studies on suitable phases for carbamate extraction have appeared [30-38].

The differences in the sorption behaviour of the carbamates on apolar, alkylbonded silica phases may cause a large variety in breakthrough volumes. Low breakthrough volumes often cause a low recovery for the more polar carbamates, *e.g.* aldicarb; methomyl and oxamyl [29] (see Table 1). As a consequence, the simultaneous determination of the oxime- and aryl N-methylcarbamates is rather difficult when using apolar phases. Although SPE with apolar phases generally provides a better reproducibility than LLE, the recoveries for the polar carbamates are lower than with LLE.

A comparative study [27] on SPE with C18- and C2-bonded silica, using aldicarb, its sulphoxide and sulphone (and 10 ml water samples), has shown that retention on the C2 phase is higher than that on the C8 and C18 phases; however the sorption of other organic material on C2 is irreversible. As a consequence, the latter phase can only be used for a few analyses, whereas *e.g.* the C8 phase did not deteriorate even after 100 analyses. From the three bonded phases, C8 generally showed the best performance, although the recovery was never over 80%.

A further comparative study on Lichrosorb RP 18 and Carboxpack B, using a variety of N-methylcarbamates (and 2 l samples), showed that Carboxpack B gives a recovery of about 90% for some of the oxime- and aryl N-methylcarbamates (oxamyl 89%, methomyl 93%, carbofuran 98% and carbaryl 96%; RSDs not reported). The extraction time could be significantly reduced by applying a flow-rate of 150-160 ml.min⁻¹ (65 x 14 mm I.D. column); However, the use of Carboxpack B resulted in contaminated blanks, and extensive conditioning of the sorbent was therefore necessary. Besides, Carboxpack B does not lend itself to the analysis of large numbers of samples [31].

In another study, cartridges containing a 'low carbon' Lichrosorb RP18 sorbent (C18/OH) were tested, using a variety of carbamates (and 50 ml samples) [32]. All compounds tested gave recoveries of 80-100 % (RSDs 1-7% at the 0.1 µg.l⁻¹ level), except for butocarboxim sulphoxide (76%), ethiofencarb sulphoxide (55%) and thiofanox sulphoxide (42%). The low recovery of the latter three compounds remains unexplained and contrasts with the satisfactory recovery with Lichrosorb RP8, and with RP18 [32]. The C18/OH phase has a retention capacity comparable to that of Carboxpack B but, fortunately, contamination is much less of a problem.

In subsequent studies, various parameters which influence the preconcentrations step, *e.g.*, rate of sample loading, particle size of the sorbent and amount of sorbent needed, were investigated. No significant influence of the sampling rate was found; small particles ($\leq 10 \mu\text{m}$) and minimal amounts of sorbent are to be preferred [33-36].

On-line coupling of SPE with LC, employing so-called membrane extraction disks and desorption by the LC eluent, has been reported recently [38-40]. If a sufficiently sensitive mode of detection is used, sample volumes can be reduced dramatically (*cf.* [32]), and breakthrough is largely prevented: on-line extraction of 10 ml samples using these disks, and LC combined with on-line hydrolysis, derivatization and fluorescence detection (*cf.* [32]) gave good results (recoveries 80-100%, RSDs 3-6%) for the oxime N-methylcarbamates [38,39]. With the on-line set-up, extraction of large sample volumes (150 ml) resulted in good recoveries (70-90%, RSDs 5-10%) for some carbamates and in breakthrough of some of the more polar analytes [40].

The selection of either LLE or SPE should primarily be based on performance, *i.e.* on recovery and reproducibility. Relevant performance data for various N-methylcarbamates are given in Table 1. Although most official methods, *e.g.* from the National Pesticide Survey [59,60], are based on LLE procedures, these procedures generally have several drawbacks that are related to practical considerations.

Table 1 Extraction recoveries (RSDs) of 2 l water samples, employing LLE (dichloromethane) and SPE (C18 packed pre-columns) with samples containing seven oxime and aryl N-methylcarbamates and two degradation products (spiking levels 0.1 and 1 ng.ml⁻¹) [29] and if 10 ml of sample, using SPE C18 extraction disks (spiking level 0.1 ng.ml⁻¹) [39].

Compound	LLE (%)	SPE (%)	SPE disks (%)
aldicarb ^a	66 (15)	86 (14)	94 (3)
aldicarb sulphoxide	19 (18)	10 (38)	87 (6)
aldicarb sulphone	95 (17)	16 (33)	90 (4)
methomyl ^a	68 (20)	25 (44)	88 (4)
oxamyl ^a	69 (11)	26 (46)	85 (4)
carbaryl ^b	83 (12)	90 (12)	80 (5)
carbofuran ^b	83 (9)	97 (4)	95 (3)
methiocarb ^b	93 (3)	98 (8)	96 (4)
propoxur ^b	69 (16)	93 (17)	93 (3)

a) Oxime N-methylcarbamate; b) aryl N-methylcarbamate

The treatment of (typically) 1 l samples precludes on-line coupling of LLE to LC and makes the procedure laborious and time consuming. Furthermore, the formation of emulsions and the necessary solvent evaporation may cause practical problems with regard to evaporation and concentration. Moreover, the considerable volume of inflammable and sometimes toxic solvents required for LLE cause waste problems.

The main advantage of SPE over LLE is the possibility of on-line coupling to chromatographic systems. In addition, less sample is required for SPE and no solvent disposal problems occur. Both LLE and SPE require an appropriate choice of materials (solvents and sorbents). Current developments in water analysis will probably lead to the large-scale introduction of on-line SPE as a basis for validated methods.

22.1.2 *Biological and soil samples*

Extraction and clean-up of plant and soil samples is complicated by the widely varying matrix composition (often characterized by parameters such as percentage of chlorophyllic organic compounds and carotenic matter, sand and clay). In addition, it is a characteristic of solid samples that ageing may negatively influence the extraction efficiency: analytes which have been in prolonged contact with a solid matrix often are desorbed less readily than freshly applied analytes. As a consequence, the recovery of freshly spiked analytes frequently provides a completely erroneous impression. The above mentioned preventive measure of extraction directly after sampling especially pertains to soil and biological matrices.

Extraction procedures for carbamates (and their transformation products) from plant or soil matrices are mainly hampered by the presence of co-extractives. Therefore, a conventional SLE procedure, like soxhlet extraction, blending or sonication, is usually followed by one or more clean-up steps. These mostly involve column chromatography over a material, such as Florisil; gel permeation chromatography (GPC) is used for the clean-up of fruit and vegetable extracts. Alternatively, SFE may be applied; it often provides sufficient selectivity to make further clean-up superfluous. In this section the extraction and clean up of plant and soil samples will be discussed separately.

For the determination of N-methylcarbamates, plant material may be treated by SLE using blending, sonication and soxhlet extraction. Generally acetone or mixtures of this solvent with dichloromethane, methanol and/or petroleum ether are employed [58]. Although it has been stated that methanol is the most suitable solvent for the extraction of carbamates from grain because it extracts contain up to 15% more of the carbamate residues than acetone or acetonitrile [52], the presence of co-extractives, *e.g.* chlorophylls and carotenes, makes methanol less attractive. To our knowledge, no systematic studies on the extraction of other carbamates from biological material have been reported.

The separation of the analytes from co-extractives by column chromatography over Florisil, silica or aluminium oxide produces good results for most carbamates. Multiple desorption using different solvents is generally applied. When employing the oxide-types phases, clean-up recoveries are generally bad (<50%) for the more polar compounds, *e.g.* aldicarb sulphoxide and sulphone [52]. In contrast, good recoveries (70-95%, RSDs 5-9%) were obtained for the simultaneous determination of aldicarb, its oxide and sulphone, if these compounds were targeted [47]. The use of aminopropyl-bonded silica column [50,51], with dichloromethane as the extraction solvent, showed good recoveries (85-100%, RSDs 2-4%) for all carbamates tested, although the recoveries of the total pre-treatment procedure did not exceed 80% for the polar compounds (aldicarb sulphoxide, butocarboxim sulphone, 1 α -naphthol and oxamyl). The advantage of aminopropyl-bonded silica over the oxide-type phases is that small extraction volumes can be used and the number of desorption steps can be kept low.

GPC of methanol extracts from blended plant material was reported to give good results with low-pigment material (apples, potatoes), whereas high-pigment materials (cabbage) required a second separation step. Such a second step was realized by

connecting a polypropylene mini-column, packed with 0.5 g Nuchar-Celite, on-line with the GPC column. Recoveries for both procedures (GPC only or CPC/N Nuchar-Celite) were comparable. Recoveries of 85-100% were obtained for some ten N-methylcarbamates, whereas the recovery for aldicarb sulphoxide was 50% [52]. Unfortunately, GPC can only be used off-line.

Soil can often conveniently be subjected to soxhlet extraction, using various solvent mixtures, and subsequent clean-up over Florisil [53-57]. A comparative study showed that acetone-dichloromethane 50:50 (v:v) gave the best extraction results for oxamyl, carbaryl, carbofuran, propoxur, 1 α -naphthol, chlorpropham and pirimicarb [57], while aldicarb and its oxidation products could successfully be extracted (85-90% recovery) in two successive steps, with acetone-water (40:60, v/v) and methanol-water (50:50, v/v) respectively [56]. In contrast to findings for plant material [52], methanol was shown to extract all carbamates insufficiently from soil samples [57]. So far, no single solvent mixture has been shown to extract carbamates and their degradation products with nearly equal and good efficiency.

Table 2 Recoveries of clean-up of some carbamates over Florisil using acetone-hexane mixtures [57].

Compounds	Acetone-hexane (v/v)		
	15:85	50:50	40:60
carbaryl	84	109	91
carbofuran	82	94	86
chlorpropham	89	101	101
1-naphthol ^a	92	40	99
oxamyl	≤ 2	73	93
pirimicarb	n.d. ^b	59	111

a) Degradation product of carbaryl; b) n.d. = not detected.

Clean-up of soil extracts is commonly performed by column chromatography. The use of Florisil and amionopropyl-bonded silica gives good recoveries (75-100%, no RSDs reported) for all carbamates, including the oxidation products of aldicarb [53-57]. Typical results [57] for Florisil clean-up are presented in Table 2. It should be emphasized that the elution of all compounds requires more than one solvent mixture and that, so far, no procedure for the simultaneous clean-up of all carbamates (and their transformation products) has been reported.

A major advantage of SFE over SLE is the fact that clean-up can be omitted if the extraction parameters are chosen to provide maximum selectivity. SFE with carbon dioxide (typical condition: 50 °C, 250 atm, 15 min) has successfully been applied to the extraction of aldicarb and carbaryl from liver samples (recoveries, 75-100%, no RSDs reported [61]) and methomyl and methiocarb from fruit and soil samples (recoveries, 100% no RSDs reported [62]). Despite these promising results, no reports have appeared on SFE treatment of soil samples yet. However, the obvious potential of the method justifies expectations that SFE will be used to a wider extent for carbamate extraction from soil and plant materials in the near future.

22.2 Column chromatography

Analytical procedures for the determination of carbamates generally involve a column chromatographic step. Initially gas chromatography (GC) was used, but nowadays column liquid chromatography (LC) is generally preferred. Supercritical fluid chromatography (SFC) is occasionally used as separation technique.

22.2.1 Gas chromatography

Many column types, detection techniques and derivatization reagents have been studied for the determination of carbamates and their degradation products [64-99]. The main problems of GC analysis of carbamates [64-93], already recognized in the early days of carbamate analysis, are thermal decomposition of the analytes on the column [63] and/or in the injector [66,69] and insufficient sensitivity of detection. Nevertheless, GC may well be the method of choice for the determination of specific carbamates or carbamate oxidation products.

Carbamates generally show a low response to electron capture (ECD), flame ionization (FID) and thermal sulphur (TSD) types of detection. Therefore, analyte detectability was initially improved by converting the carbamates into, *e.g.* the N-perfluoroacyl [74], N-perchloroacyl [77] or pentafluorobenzyl [79] derivatives for ECD detection. Later, with the advent of nitrogen/phosphorus (NPD) and mass spectrometric (MS) detection, such derivatization methods became obsolete.

Thermal degradation is a key problem in the GC analysis of carbamates. For example, N-methyl and N-phenylcarbamates are labile at higher temperatures ($>100^{\circ}\text{C}$), whereas N,N-dimethylcarbamates, such as pirimicarb, are not subject to thermal degradation under GC conditions. Thermal degradation may be prevented by the use of special GC systems or by appropriate derivatization. Alternatively, derivatization may be required to yield more volatile derivatives for degradation products such as amino hydroxypyrimidine compounds (from pirimicarb), because these compounds are not directly amenable to GC. Derivatization and instrument modifications as a means to prevent thermal degradation are discussed below.

In the past, insufficiently deactivated column material caused degradation with a variety of packed-column stationary phases (Carbowax 20M, polar; Apiezon N, non-polar; SE 30 & QF 1, mixed polarity [63]). Extensive deactivation of the stationary phase and the column support material, and the exchange of a glass injection liner by quartz, were shown to suppress decomposition [64]; however, decomposition tended to increase upon column ageing. The introduction of high-quality, deactivated capillary columns (SE-54 [69]), helped to prevent thermal degradation on the column and effectively limited thermal degradation to the injector. Despite the fact that it has been shown that contamination of the injection port due to poor clean-up of extracts promotes the degradation of carbamates [100], thermal lability is an intrinsic property of some carbamates. Recently, the heating of compounds in the injector was successfully avoided by the application of programmed temperature vaporization (PTV) and cold on-column injection for the GC analysis of some thermolabile aryl N-methyl-carbamates [67-69] (note that the injector inlets and packings still required deactivation). Fig. 2 shows some illustrative chromatograms.

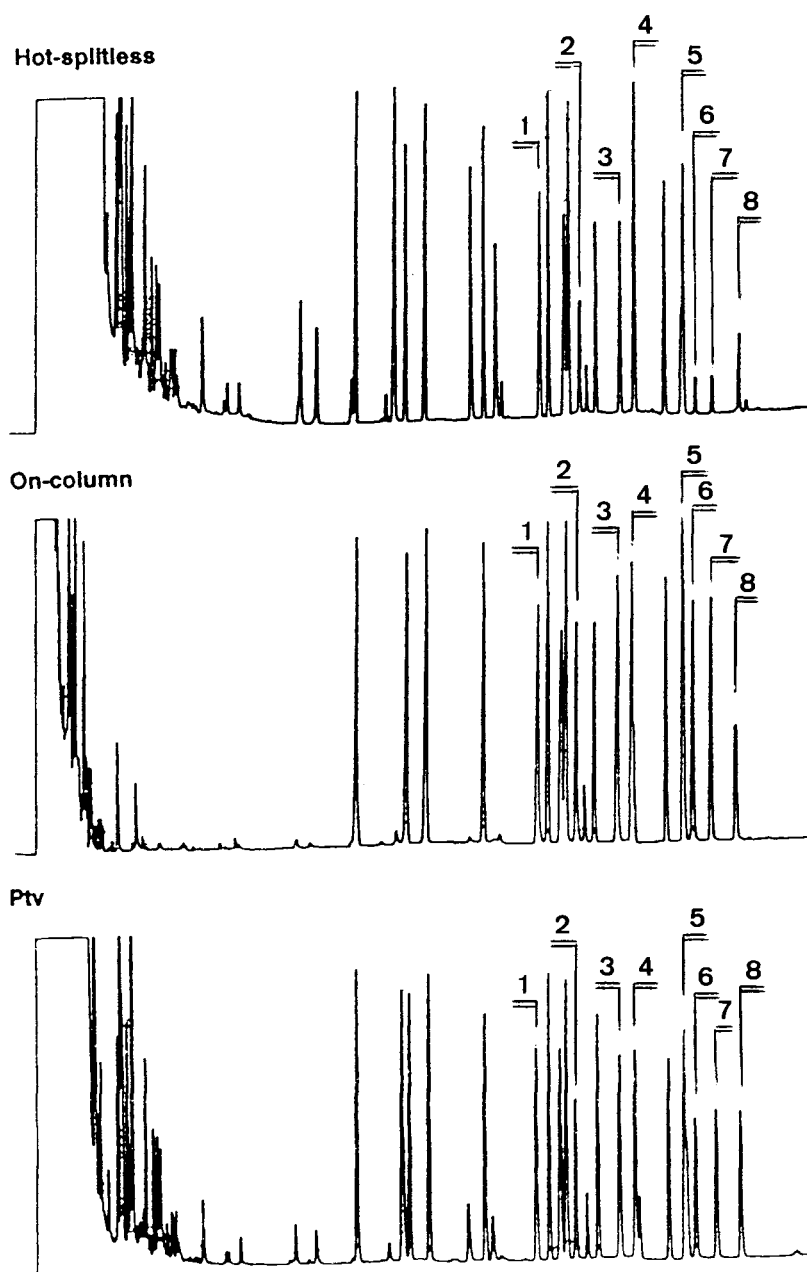


Figure 2 GC-NPD chromatograms of eight aryl N-methylcarbamates and one N,N-dimethyl carbamate (pirimicarb), using hot splitless (top), cold on-column (middle) and programmed temperature vaporizer (bottom) injection [69]. Peak assignment: 1: propoxur, 2: bendiocarb, 3: carbofuran, 4: aminocarb, 5: pirimicarb + ethiofencarb, 6: dioxacarb, 7: carbaryl, 8: methiocarb.

The prevention of thermal degradation by derivatization is mainly used for aryl N-methylcarbamates, which can easily be hydrolysed (to give phenol-type products) and subsequently converted to thermally stable esters [63]. This kind of derivatization may lead to serious overestimation of pesticide levels because the natural, phenol-type degradation products are also derivatized. As an alternative means of derivatization, it has been shown for methomyl (an oxime, N-methylcarbamate) that hydrolysis to the oxime and subsequent derivatization of this moiety provides a useful conversion to thermally stable compounds [90]. Although derivatization of the carbamates is not generally applicable, it may be of use for target compound analysis.

Despite the above mentioned thermolability problem, some GC/MS methods using chemical ionization MS (CI-MS with isobutane as the reagent gas) have been reported for selected compounds [92-99]. Six GC stationary phases were tested in packed columns, in combination with CI-MS detection, for 32 carbamates [172]. Moderate column temperatures ($<185^{\circ}\text{C}$) and short analysis times allowed detection of intact carbamates, except for carbaryl (ca. 50% degradation), from spiked soil sample extracts (recoveries 80-112%, RSD 2-9%, carbamates) [181]. Packed-column GC/CI-MS was used for aldicarb and butocarboxim and their metabolites [173,174]. Low column temperatures ($\leq 150^{\circ}\text{C}$) led to successful identification of the intact compounds, but no quantitation was reported [182,183]. Short capillary columns and CI-MS detection were also used to overcome thermolability problems [184,185]. In both cases, GC with CI-triple quadrupole MS/MS detection as used, either to facilitate identification from the CI spectra [184] or to gain selectivity in compensation for loss for chromatographic resolution [185]. no quantitative data are reported in the latter study (on aldicarb, its sulphoxide and sulphone), but the former study specifies absolute LODs of 0.15 ng (aldicarb nitrile), 0.3 ng (aldicarb) and 1.2 ng (aldicarb oxime) with a linear response from the LOD up to 150 ng and in full-scan mode [185].

Although some compounds, particularly aldicarb, its sulphoxide and sulphone are definitely not amenable to GC, GC with CI-MS detection (and MS/MS) may lead to unequivocal identification and low limits of detection for some carbamates. In other words, although GC is not the method of choice for the simultaneous determination of a wide variety of carbamates and their degradation products, GC with CI-MS detection, possibly combined with derivatization, is a powerful method for target analysis.

22.2.2 Column liquid chromatography

The application of LC for the separation of carbamates has evolved over the last two decades. A 1975 review on the determination of carbamates [94] signalled the potential of LC as compared to GC: LC generally requires less extensive sample pretreatment and enables on-line trace enrichment and sample clean-up. This review was followed by a comparative study on the separation of carbamates [101], using various normal-phase and reversed-phase modes of operation, and by a report on pesticide analysis [102], which gave LC experimental conditions for many carbamates. Several reports on the target LC analysis of carbamates and their degradation products have been published since [103-109]. The quality as well as the nature of the stationary phase are key parameters in most LC studies and both subjects are discussed below.

The presence of free silanol groups may lead to bad peak shapes, especially for the polar carbamates and their transformation products. This has been demonstrated for the simultaneous separation of aldicarb and its oxidation products, and their oximes [105-

110], pirimicarb and three of its degradation products [111] and for oxamyl [57]. In all cases the use of a buffer in the LC eluent or of a specially end-capped phase [109] was necessary to maintain separation and constant retention times with alkyl-bonded silica phases. Unfortunately, end-capped phases tend to be lost during use [111] and the separation capacity can be maintained by using buffered eluents. However, the use of buffered eluents has been shown to result in irreproducible retention times for the polar carbamates [105]. Regular monitoring of the separation quality and the retention times is therefore recommended.

One study [101] on the use of a wide variety of phases (silica, cyano-bonded, amino-bonded and C18-bonded silica and ether phase-ETH), showed that most compounds could be separated on silica and cyano-bonded silica, whereas amino-bonded silica gave peak broadening, especially for the more polar compounds. In the reversed-phase mode C18-bonded silica gave the best results [101]; no separation of the polar compounds was achieved on ETH. 22 carbamates could be separated on C18, using gradient elution (20 to 60% acetonitrile in water). Another comparative study [103] showed that Zorbax C8 and Zorbax CN both give insufficient separation: the former did not produce baseline separation of propoxur, carbofuran, bendiocarb, carbaryl and 1 α -naphthol, while the CN phase did not separate aldicarb sulphone and oxamyl (Fig. 3).

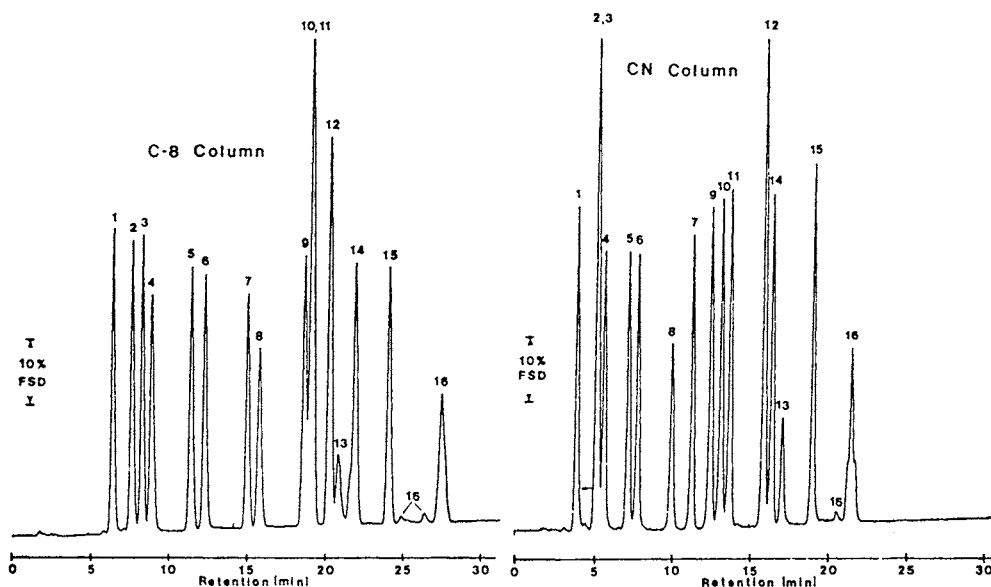


Figure 3 LC with fluorescence detection, using post-column derivatization, of ten carbamates and six degradation products, obtained with (A) C8 and (B) CN phases, using a 25 cm, 4.6 mm i.d. Zorbax column (6 μ m spherical particles) and a 30 min linear gradient from 12 to 70 % acetonitrile in water; flow-rate, 1.5 ml.min⁻¹). Column temperature, 30 °C [103]. Peak assignment; 1) aldicarb sulphoxide, 2) aldicarb sulphone, 3) oxamyl, 4) methomyl, 5) 3-hydroxycarbofuran, 6) methiocarb sulphoxide, 7) methiocarb sulphoxide, 8) aldicarb, 9) propoxur, 10) carbofuran, 11) bendiocarb, 12) carbaryl, 13) 1 α -naphthol, 14) landrin, 15) methiocarb, 16) bufencarb.

Incomplete separation of pirimicarb and its seven degradation products on polar Hibar RP8 and amino columns in the reversed-phase mode has also been reported [104]. A comparative study of several phases [110] showed that aldicarb and five degradation products are best separated on phenyl- and cyano-bonded phases. Obviously, available LC phases have a limited capacity for the separation of mixture of carbamates [32] and their degradation products, with problems especially being encountered for polar compounds.

22.2.3 *Supercritical fluid chromatography*

The potential of SFC is generally intermediate between those of GC and LC; in other words, where GC is subject to problems because of the thermolability of compounds and LC lacks separation power and/or sensitive detection, SFC offers good perspectives. However, only a few reports on SFC (packed SFC in one case [116]) have appeared for carbamates, with detection by means of multichannel UV [112], FID [113] and MS [114-117]. The potential of SFC is nicely demonstrated by the fact that nine carbamates and degradation products were completely separated at 100 °C within 4 minutes [117]; thermal degradation of the carbamates will probably start to occur at higher temperatures. Although the only detection limit reported (ca. 40 mg.l⁻¹ of bendiocarb; UV detection at 200 nm [112]) is not too encouraging, the separation power and the analysis time of SFC may well be beneficial in solving certain problems.

22.3 LC detection methods

As stated above, LC is often preferred for the separation of thermolabile and/or non-volatile carbamates. It is usually combined with UV [118-140], electrochemical (ECD) [141-150], fluorescence [151-175] or MS [176-216] detection. Analyte detectability and selectivity are the most important characteristics to be discussed below.

22.3.1 *Ultraviolet detection*

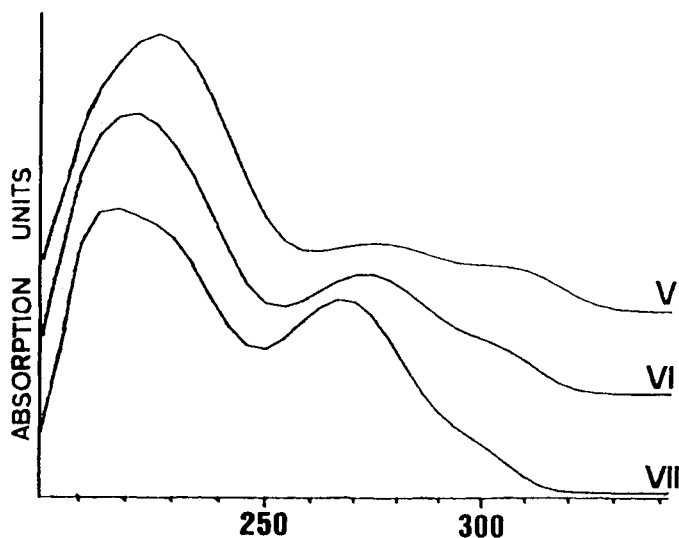
Monitoring UV absorbance at properly selected wavelengths and/or recording UV absorption spectra, if diode array detection (DAD) is applied, provides a convenient means of detecting most organic compounds. Many carbamates have a UV absorption maximum in the range of 190-225 nm, and additional maxima at wavelengths between 245 and 280 nm [24,96]. Relevant data are shown in Table 3. It is obvious that multiple-wavelength detection is required to obtain near-maximum sensitivity if a large number of analytes has to be determined.

UV detection can best be combined with acetonitrile/water mixtures as an eluent, because this allows detection at 195-200 nm (methanol/water at 205-210 nm and tetrahydrofuran/water at >220 nm). Interferences may, of course, be caused by compounds which strongly absorb at 190-220 nm, e.g. humic acids; in many LC separations a broad hump occurs at low retention times. It has been stated that such interferences may efficiently be trapped by using cyanopropyl-bonded silica pre-column [111].

Table 3 UV absorption maxima (λ_{max}) and extinction coefficient ($\epsilon_{\text{max}} \times 10^3$) of seven carbamates and two transformation products in acetonitrile [101].

Compound	λ_{max} (ϵ_{max})	λ_{max} (ϵ_{max})	λ_{max} (ϵ_{max})
aldicarb	193 (40.8)	248 (1.9)	
aldicarb sulphoxide	193 (27.9)	248 (1.9)	
aldicarb sulphoxide	199 (11.0)		
barban	206 (41.1)	238 (16.8)	278 (1.1)
carbaryl	222 (86.3)		281 (6.3)
carbofuran	200 (47.8)	218 (6.3)	280 (3.0)
methiocarb	202 (55.6)	220 (12.3)	266 (2.8)
methomyl		233 (9.1)	
propoxur	195 (58.6)	215 (7.6)	278 (1.5)

Generally speaking, UV detection for carbamates has average sensitivity, with LODs in, typically, the lower ng range (cf. [126]). In systems involving on-line trace enrichment this may still be sufficient for, e.g., surface water analysis with diode array detection and will provide some structural information. However, one should be aware of the limitations of this technique, as is nicely illustrated in Fig. 4 for the pirimicarb metabolites V, VI and VII. One should also be aware of problems encountered with complex samples - or, in other words, insufficient clean-up - which may explain reported differences in LODs of carbamates by one order of magnitude when comparing water and soil samples [128,125].

**Figure 4** DAD-UV absorption spectra of the metabolites V, VI and VII of pirimicarb.

In conclusion, for most sample types LC with UV detection is suitable for the trace level determination of carbamates only if it is combined with concentration and/or elaborate clean-up procedures.

22.3.2 *Electrochemical detection*

Despite the inherent sensitivity and selectivity of electrochemical detection (ECD) it has not been as extensively used for LC as might be expected. Problems encountered with the robustness of LC-ECD systems (electrode materials electrode fouling, required experience) probably are the main causes. Analyte detectability in LC-ECD is strongly influenced by, e.g., electrode geometry and nature [141,143,145], the LC eluent composition (especially the pH; e.g. for carbaryl [141], and the presence of electro-active co-extractives.

LC-ECD of most aryl, N-methylcarbamates and N-phenylcarbamates can easily be performed because these compounds can be oxidized within the available range. For example, aminocarb can be detected at a concentration as low as $5 \mu\text{g.l}^{-1}$ [141]. The use of a detection potential of about 1.1 V is commonly considered best, although the oxime, N-methylcarbamates and some aryl, N-methylcarbamates (carbaryl and carbofuran) are electro-inactive at this potential. Despite the practical limitations due to the oxidation of water, ECD at 1.9 V has been reported for some N-methylcarbamates (aldicarb, its sulphoxide and sulphone, amionocarb, bendiocarb, carbaryl, methiocarb and methomyl); unfortunately, the LOD and oxidation potential were specifically mentioned for aminocarb only [144]. Alternatively, it was shown that aryl, N-methylcarbamates, e.g. carbaryl and carbofuran, can be hydrolysed on-line to the corresponding phenols, which can subsequently be oxidized at potentials of 0.5-0.8 V [146,147]. The general tendency that lower potentials lead to a lower background current and, thus, to better limits of detection is illustrated by the LODs of 10-35 $\mu\text{g.l}^{-1}$ obtained for some aryl, N-methylcarbamates [147]. For the rest, it has been observed that LODs for ECD are one order of magnitude better than those obtained with UV detection; however, the reproducibility of ECD is less than that of UV detection; this is probably due to the slowly increasing contamination of the electrode surfaces referred to above [141-147].

From the above it will be evident that LC-ECD is mainly useful for target analysis of aryl, N-methyl- and N-phenylcarbamates.

22.3.3 *Fluorescence detection*

The number of compounds that display native fluorescence is rather restricted. As a consequence, fluorescence monitoring is a selective - and also often highly sensitive - method of detection in LC. The selectivity of fluorescence over UV absorption detection is further improved by the fact that two (excitation and emission) rather than one (absorption maximum) wavelength(s) have to be selected. Compounds which are not fluorogenic, such as most carbamates, can only be detected if they are derivatized or otherwise chemically converted into highly fluorescent products.

Fluorimetric detection of carbamates was already used in the sixties, either for direct analysis (e.g., carbaryl) or for the analysis of derivatives (e.g., benomyl) [151,152]. The determination of carbamates and their degradation products by a combination of fluorogenic labelling and LC was first demonstrated in 1974 [153]. Pre-column derivatization with dansyl chloride and subsequent normal-phase separation gave detection limits of 0.5-2.5 mg.l^{-1} [153]. The method was successfully applied to the determination of carbaryl in potato and corn samples [154]. On-line post-column

derivatization, using ortho-phthalaldehyde (OPA) as the reagent, was first reported for carbamate analysis in 1977 [155]; a scheme of the on-line analytical system is given in Fig. 5.

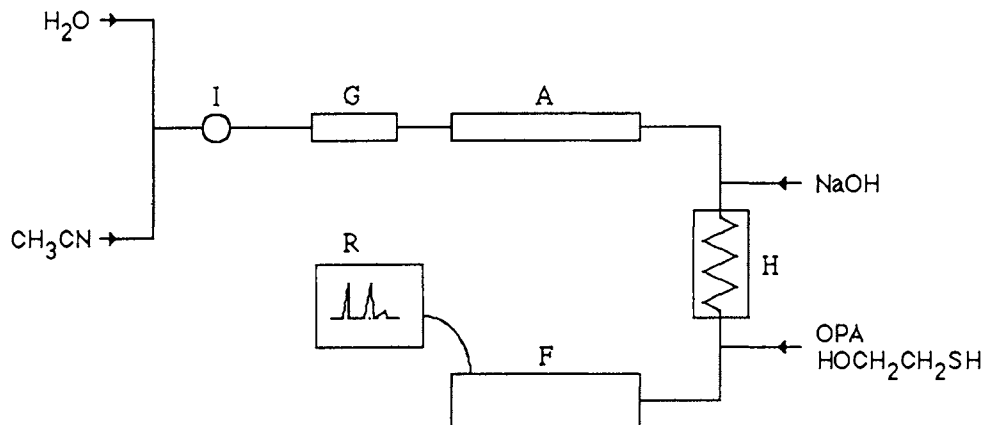


Figure 5 Scheme of an LC-hydrolysis-fluorescence detection system for N-methylcarbamates; A: analytical column, F: fluorescence detector, G: guard column, H: hydrolysis chamber, I: injector, R: recorder [155, 158, 160].

The OPA derivatization is preceded by alkaline hydrolysis to convert the N-methylcarbamates to methylamine, which is subsequently reacted with OPA in the presence of a reducing agent such as 2-mercaptoethanol, and the resulting fluorophore is monitored. Several important parameters have been studied and the reaction conditions were optimized [156-161]. The OPA derivatization finally formed the basis for the reference method (for N-methylcarbamates) of the U.S. EPA [40] and was commercialized by Perkin-Elmer and the Pickering Laboratories. One main advantage should be mentioned here; OPA itself is completely non-fluorescent - in other words, there is no need to remove the (large) excess of reagent after post-column reaction, i.e. prior to the actual detection.

The initial set-up of the OPA post-column reaction detection system had some disadvantages. Peak broadening occurred, due to the extra dead volume generated by the reaction coils and tee-pieces (reagent delivery for two reaction steps), the hydrolytic conversion was not complete and reproducibility deteriorated over time due to the limited stability of 2-mercaptoethanol. As an alternative, catalytic conversion and heating [161], using a reactor packed with a basic anion-exchange resin [162-164] or magnesium oxide [165], proved to be successful. Although the rate of hydrolysis of the N-methylcarbamates differs widely, with temperature optima from 90-100 °C to over 140 °C [162], limits of detection as low as 0.1 ng (aldicarb, and 0.85 ng (methiocarb) were reported for the modified system [162]. Miniaturization of the reactor, to achieve compatibility with narrow-bore LC, led to even lower LODs [166]. More recently, photolytically initiated hydrolysis was introduced, which makes N,N-dialkylcarbamates accessible to reaction with OPA. Photolytic hydrolysis sometimes requires the addition of sensitizers (e.g. acetone) to the eluent to effect the conversion of e.g. aldicarb

sulphone. Typical LODs of $2.5 \mu\text{g.l}^{-1}$ were reported [167,168]. Further optimization of the reactor geometry and of the solvent conditions allowed a one-step conversion of the carbamates to the OPA derivatives [157], with similar LODs. Finally, the use of 3-mercaptopropionic acid and N,N-dimethyl-2-mercaptoethylamine hydrochloride ('thiofluor') reagents instead of 2-mercaptoethanol greatly improved reproducibility over a longer time period [170]. The combination of this analytical method with on-line pre-column extraction and SPE-based trace-enrichment resulted in detection limits of 0.02-0.03 $\mu\text{g.l}^{-1}$ for all N-methylcarbamates, and some of their degradation products, in water samples [32]. The application of this method to fruit samples was optimized and validated [51].

In conclusion, on-line post-column OPA derivatization is an excellent tool for the determination of a large number of, though not all (e.g., N-phenylcarbamates), carbamates. Being essentially a derivatization-type procedure, it can not be used for degradation products which do not yield a primary amine upon hydrolysis, nor does it provide structural information. Even so, it is possibly the best procedure available today for the trace-level analysis of carbamates.

22.3.4 Mass spectrometric detection

Mass spectrometry (MS) may be considered as a nearly universal method of detection, since gas-phase ions can be generated from most compounds. The high-vacuum system of a mass spectrometer (typically operated at pressures $<10^{-5}$ mBar) was successfully coupled to GC in the late sixties, and to LC and SFC in the early eighties. Modern GC/MS instruments generally use capillary column GC and direct introduction of the column effluent into the MS source (operation either under electron ionization, EI, or under chemical ionization CI, conditions).

Commercially available GC/MS is routinely used as a tool for compound identification (typically at the high $\mu\text{g.l}^{-1}$ level) and of compound quantitation (typically at the low $\mu\text{g.l}^{-1}$ level). Modern LC/MS cannot be described in general terms, because there are different methods of interfacing LC to MS. This section discusses the use of LC/MS for the determination of carbamates and their degradation products. As regards ionization methods, EI mass spectra of carbamates generally produce most information and allow unequivocal identification, if necessary with the help of spectrum libraries. CI mass spectra of carbamates give molecular weight and some structural information. Spectra from desorption ionization, e.g. fast atom bombardment (FAB), and from LC/MS-related methods of ionization (thermospray, electrospray) produce CI-like spectra.

The EI mass spectrometry of N-methylcarbamates was reviewed in a book on environmental analysis [170]. Most N monosubstituted carbamates show a molecular ion, $[\text{M}]^+$, whereas at least one characteristic fragment ion is usually observed: the loss of neutral alkyl- or arylisocyanate produces the alcohol radical cation, $[\text{M} - \text{RNCO}]^+$. The EI spectra of the N,N-dimethylcarbamates, as well as those of most degradation products, cannot be described in general terms.

The positive ion CI (PCI) mass spectra of carbamates generally show signals of a protonated molecule, $[\text{M} + \text{H}]^+$, and of the protonated alcohol, $[\text{M} + \text{H} - \text{RNCO}]^+$ [177-181]. The loss of CH_3NCO from protonated N-methylcarbamates has been investigated in some detail by deuterium labelling and MS/MS experiments [179-181]. The intensity ratio of the $[\text{M} + \text{H}]^+$ and $[\text{M} + \text{H} - \text{RNCO}]^+$ signals varies with the reaction gas used. Moreover, some carbamates show adduct ion signals, $[\text{M} + \text{NH}_4]^+$, if ammonia is used as the reaction gas. In general, carbamates have a proton affinity close to that of ammonia

and protonation should therefore result in good detection sensitivity.

The negative ion CI (NCI) mass spectra of carbamates have been less well studied. Studies [182-185] show that molecular anions, M^- , are only observed with some halogen-containing carbamates and that deprotonated molecules have a low abundance or are completely absent. Typical negative fragment ions are the alcoholate ions, $[RO]^-$, and the carbamic acid anion, *e.g.* $[CH_3-NH-C(=O)-O]^-$ from N-methylcarbamates) [183-185]. Deprotonation and negative fragment ion formation is inefficient for most carbamates, giving rise to poor detection sensitivity. Deprotonation is potentially more efficient for the degradation products, *e.g.* the phenol-type compounds, and better sensitivity is to be expected for these compounds. The general characteristics make NCI less suitable for carbamate analysis.

FAB ionization [186], as well as LC/MS-related ionization (see below) of the carbamates, produces CI-like spectra, which typically exhibit the loss of $RNCO$ from $[M+H]^+$ with positive ion detection. FAB has been applied to the specific detection of benomyl, a compound that is known to decompose to carbendazim under all other (Ms) condition [186]. FAB ionization, and any of the other desorption-type ionization techniques, are of very limited use for the determination of carbamates because quantitation is extremely difficult, if not impossible, and because CI produces essentially the same information with less practical limitations.

As regards the potential of LC/MS, LC provides adequate separations and MS has the capability of detection and identification of trace amounts of compounds (typically at the ng level). This was recognized early in the development of LC/MS interfacing and, consequently, reports have appeared on carbamate analysis using various interfaces: moving belt (MB) [187-189], particle beam (PB) [183-185, 190-192, 210], direct liquid introduction (DLI) [193, 194], thermospray (TSP) [30, 182, 195-206, 210], electrospray (ES) [207], ionspray (ISP, also termed 'nebulizer assisted ES') [208, 210] and atmospheric pressure chemical ionization (APCI) [209, 210]. In addition, LC/MS/MS has been used for carbamate analysis [211]. Recently, PB, TSP, ISP and APCI were compared in one study, using eight selected carbamates [210]. In the following the impact of these interfacing methods on carbamate analysis will be briefly evaluated.

Only the MB and the PB types of interface are capable of (nearly) full desolvation upon transfer of the analyte from the LC effluent to the MS high vacuum. In principle, this allows operation of the mass spectrometer with common MS ionization modes, EI and CI (eluent independent). However, MB requires heating during desolvation and may therefore induce thermal degradation of the carbamates. Other general, practical flaws of MB, *e.g.* severe memory effects, make the interface even less suitable for the analysis of carbamates. PB interfacing generally has poor sensitivity (typically at the $mg.l^{-1}$ level) compared with the other interfacing methods. The detection sensitivity was shown to decrease if more organic modifier is used in the mobile phase [213]. The LC-to-MS transfer efficiency of analytes with PB is concentration-dependent [192, 214] and calibration curves are therefore not linear over a large range. The fact that linear response curves have been reported for oxamyl in a concentration range of 4-80 $mg.l^{-1}$ [190] shows that non-linearity probably occurs at concentrations below the $mg.l^{-1}$ level (note that 4-80 $mg.l^{-1}$ is amenable to UV detection). It has been claimed, but not convincingly demonstrated, that linear calibration curves at low concentration can be obtained by adding a 'carrier compound' to the LC eluent [192, 215, 216]. The relatively low sensitivity of detection may be improved by sample preconcentration, using on-line SPE, thus allowing LC/PB-MS identification from EI mass spectra at low $\mu g.l^{-1}$ levels [216].

DLI, TSP, ES, ISP and APCI typically yield CI-like mass spectra with the solvents and additives acting as the reagent gas. In addition, ion/molecule complex formation of the analyte and eluent components is frequently observed. However, the mass spectra obtained with each of these interfacing methods differ widely, as is illustrated for carbofuran in Table 4. This is generally attributed to the fact that many parameters influence the ion formation and, consequently, the efficiency of detection. LC eluent composition, source temperature, source pressure ionization assistance (e.g. by discharge), eluent flow rate and MS source tuning should be optimized to produce maximum sensitivity. Optimization of the experimental conditions often requires compromises, since most parameters are interdependent and many optima differ for different compounds.

Table 4 Ions reported for carbofuran, when using various MS ionization methods.

Ionization (specification)	Ions, m/z (relative abundance %) ^a	Reference
CI (NH ₃ reagent)	222 (100), 165 (11)	179
CI (CH ₄ reagent)	222 (24), 165 (100)	179
CI (NH ₃ reagent)	239 (20), 222 (100), 182 (5), 165 (20)	117
CI (CH ₄ reagent)	222 (40), 165 (100)	117
PB-CI (NH ₃ reagent)	222 (100), 165 (100)	210
DLI (CH ₃ CH/H ₂ O eluent)	263 (?), 222 (?), 165 (?)	193
DLI (CH ₃ CH/H ₂ O eluent)	263 (4), 222 (100), 206 (5)	194
TSP	239 (38), 222 (100)	29
TSP	239 (100), 222 (67)	195
TSP	239 (?), 222 (100)	211
TSP	280 (18), 254 (10), 239 (55), 222 (100)	198
TSP	239 (100), 222 (40)	202
TSP	239 (100), 222 (25)	210
ISP	222 (100), 165 (24)	210
APCI	222 (100), 165 (64)	210

a) Abundance relative to base peak; (?) denotes that ion was reported but no abundance was given.

Although DLI provides satisfactory LC/MS interfacing, sensitivity in carbamate analysis [193,194] and, indeed, in most analyses, is low. Typical LODs of 40-100 ng [193] and 20 ng - 40 µg [194] were reported for 11 carbamates using full-scan detection. The sensitivity is low because the flow rates permitted (typically 10-30 µl.min⁻¹) require post-column splitting (no reports on microbore LC/CLI-MS of carbamates have appeared). Probably, the need to split has precluded research on continuous-flow FAB of the carbamates. DLI has been superseded by TSP, ES, ISP and APCI. Because these latter techniques produce essentially similar information, no gain is to be expected from a combination of preconcentration techniques with DLI.

TSP is the most popular interfacing technique, although the chemistry of ion formation under TSP conditions is not yet well understood. The interface geometry is completely different from the DLI geometry and TSP does not require splitting because the excess solvent is largely removed by increased pumping capacity. TSP-MS places some restrictions on the chromatographic system (non-volatile salts or reagents should not be present), but it may conveniently be performed in the presence of an ionogenic compound (typically ammonium acetate) [195]. This additive is required to induce proton transfer reactions during, and after evaporation, thus causing the formation of detectable ions. In the PI mode, protonation and ammoniation of the analyte are typically observed (giving rise to $[M + H]^+$ and $M + NH_4]^+$ ions, respectively). In the NI mode, electron capture, deprotonation and acetate adduct formation are commonly observed giving rise to M^- , $[M - H]^-$ and $[M + CH_3COO]^-$ ions, respectively).

Most carbamates are amenable to TSP-MS detection, despite the thermal character of the desolvation process [182]. Detection of carbamates is generally most efficient in the PI mode, while the NI mode is best for detection of halogenated carbamates and hydroxyl-containing degradation products. The performance of TSP-MS is improved, at least for carbamates, if the ionization is assisted either by using a discharge or by a filament [29,182,195-206,210]. Under all TSP conditions the carbamates tend to fragment by losing CH_3NCO from protonated molecular ions or even from adduct ions. More specific fragmentation may be obtained from MS/MS experiments, using *e.g.* a triple quadrupole MS [211], but MS/MS techniques generally cause analyte detectability to deteriorate. Furthermore, many carbamates tend to form adduct ions, with ammonia or with solvent molecules, or (apparently) with 'odd' ions, *e.g.* $[M + 32]^+$ and $[M + 59]^+$ [198,206]. Whereas fragmentation may provide limited means of structure attribution, the occurrence of 'odd' and adduct ions may hamper identification. Moreover, fragmentation, 'odd' ion and adduct ion formation represent side reactions which may be undesirable from the point of view of quantitative analysis.

The parameters which characterize TSP conditions have been studied extensively for carbamates [182,204,205]. Instrument parameters, specifically the geometry of the TSP interface and the MS source, are (generally) invariant parameters, which may be of major influence. With filament or discharge assisted ionization and PI detection being most effective, ion source temperature, vaporizer temperature and eluent composition are important optimizable parameters, which may exhibit different optima for different instruments.

It was shown that high source temperatures (up to 300 °C) lead to more intense fragment ion signals ($[M + H - CH_3NCO]^+$ and $[M + NH_4 - CH_3NCO]^+$) with asulam, oxamyl, chlorpropham, desmedipham and phenmedipham [204]. This increase in intensity is probably due to thermal dissociation by loss of CH_3NCO , with subsequent protonation (ammoniation) of the alcohol-type reaction product, since near thermal equilibration conditions occur inside the source under the high pressure applied (estimated at $<10^{-1}$ Torr). Hence, the source temperature for detection of the carbamates as such has a maximum. The vaporizer temperature may also induce thermal dissociation, as was shown for methiocarb [182]. This temperature is more critical, since adequate evaporation and stable ion currents are only obtained above a certain minimum temperature. Thermal dissociation may easily be established by monitoring fragment and quasi-molecular ion currents over a range of temperatures. The vaporizer temperature may well influence the degree of desolvation of the analytes, but no effects on adduct ion intensities have been reported so far.

The LC eluent composition affects the efficiency of detection, because it primarily determines the possibility for adduct ion formation, for protonation and for fragmentation [182]. Moreover, the eluent components influence the initial vapour formation and different eluents therefore generally require different vaporizer temperatures.

So far, only two reports have appeared on (nebulizer assisted) ES-MS detection of carbamates [207]. Although ES was, until recently, limited to low flow rates, typically 2-10 $\mu\text{l}\cdot\text{min}^{-1}$, low LODs (20-40 pg, 10 $\mu\text{g}\cdot\text{l}^{-1}$) [206] can be obtained. The quoted values were obtained with an ion trap type mass spectrometer. Desolvation in the electrospray is enhanced by using a counter-current nitrogen flow, which, together with the application of a voltage over certain parts of the interface ('cone voltage'), may be used to collisionally induce dissociation (CID) of the ions. Although CID yields structure-specific information, it reduces the ES sensitivity, either by a decrease of the intensity of the largest peak (with both nitrogen and voltage) or by increased 'chemical noise' (with the voltage, due to more efficient transmission of solvent cluster ions) [207]. The recent development of a high flow rate ISP interface caused the sensitivity to deteriorate compared with the low flow rate technique, probably due to less effective ionization [208]. Recent developments have led to more effective high-flow ISP and ES, e.g. by the use of ultrasonically assisted nebulization [217]. Carbamates have not yet been studied with these modified interfaces.

Although APCI has not yet been extensively studied, the preliminary results are promising. Conventional LC flow rates of 1.0 $\text{ml}\cdot\text{min}^{-1}$ can be used if thermally assisted nebulization is used. Detection limits of 50-100 $\mu\text{g}\cdot\text{l}^{-1}$ have been obtained for carbaryl, carbofuran and aldicarb [210]. Most recently, plasma induced atmospheric pressure ionization has been introduced [218,219], but this mode of ionization has not yet been applied to carbamate analysis.

As was discussed above, several available LC/MS methods have been studied for their potential with respect to carbamate analysis. So far, ES performs best for a wide variety of carbamates, since it results in detection limits in the low $\mu\text{g}\cdot\text{l}^{-1}$ range and provides direct identification. TSP and PB do not perform as well as ES, but these methods of detection may be enhanced by applying preconcentration. Future developments, specifically of high-flow ES [217] and of plasma ionization methods [218, 219], may lead to further improvements.

22.4 Conclusions

Most carbamates and their degradation products can be determined in various matrices at low (and even sub $\mu\text{g}\cdot\text{l}^{-1}$) concentration levels. The thermolability and sensitivity to hydrolysis create special problems for some carbamates. To allow an overall impression of the potential of different methods of detection, Table 5 gives typical LODs for oxime- and aryl N-methylcarbamates in aqueous samples, with or without preconcentration. It should be noted that the methods of sample preparation and of separation in the quoted papers differ widely and that the compound classes selected are not representative for all carbamates. Besides, (on-line) trace enrichment has been used in a few instances only. For aqueous samples analyte isolation is preferably carried out by (SPE), and for biological materials and soil by conventional liquid-liquid extraction. SFE is a promising method for the extraction of analytes from biological materials and soil, but more research is required. Separation of the compounds is best performed by LC on C8- or cyanopropyl-bonded silica with methanol/water or acetonitrile/water mixtures as eluent.

Table 5. Typical detection limits of oxime- and aryl N-methylcarbamates, as reported for LC with various detectors.

Detection mode	LOD ($\mu\text{g.l}^{-1}$)	Reference
UV/DAD	20-200	122
UV/DAD ^a	0.01-5	38
ECD	1-7	144, 147, 149
Fluorescence	1-10	38
DLI-MS	20-150	193
TSP-MS	40-150	210
ESP-MS	1-10	207
ISP-MS	10-100	208
PB-MS	2000-88000	191
PB-MS ^a	2-88	191
APCI-MS	50-100	210

a) combined with on-line trace enrichment.

While UV absorbance detection is the conventional choice when studying carbamates, the N-methylcarbamates can be detected with much more sensitivity and selectivity by subjecting them on-line first to hydrolysis and then to reaction with OPA to form a highly fluorescent product. LC can efficiently be combined with on-line preconcentration, which may easily improve analyte detectability 100-1000-fold in terms of concentration units. MS provides the means to confirm, and sometimes unambiguously identify, carbamates and their degradation products. LC/PB-MS, which has the best identification potential, requires substantial preconcentration if the analytes have to be detected at low levels. In most cases, LC/TSP-MS can successfully be applied, again preferably with preconcentration, for the low-level analysis of most sample types. The first report on APCI of carbamates shows that LC/APCI-MS may well become a competitor of LC/TSP-MS. The detection sensitivity of ES roughly equals that of TSP, but it is to be expected that high flow ES will yield distinctly improved results. Together with the fact that CID-ES provides better means of identification than TSP, this justifies our expectation that high flow LC/ES-MS and APCI will become the best available methods to study carbamates and their degradation products.

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23.

Method development for the determination of polycyclic aromatic hydrocarbons (PAHs) in environmental matrices

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Polycyclic aromatic hydrocarbons (PAHs) and some of their derivatives represent a class of important environmental pollutants possessing a high mutagenic and carcinogenic potential as proven by *in vitro* experiments with various animal species using different modes of application and *in vitro* experiments using tissue homogenates, cells in culture and subcellular fractions as well [1,2].

PAHs are ubiquitous in the environment, although their concentrations vary within a wide range in the various matrices that are relevant to man. They originate mainly from two sources. First, they are formed as natural side products during the coalification of biomass to fossil fuels such as peat, lignite, crude oil and hard coal. During this process preformed structures are converted into polycyclic aromatic compounds by a series of condensation, water elimination and dehydrogenation reactions [aromatization process]. Prominent examples for this process are (a) the formation of perylene from fungal and insect pigments as an early occurring PAH in peat [3]; (b) the formation of retene (1-methyl-7-isopropylphenanthrene) from abietic acid in pine-tree resins as found in soils and in lignite [4,5]; and (c) the formation of chrysene and picene derivatives from plant triterpenes as found in sediments [6].

Apart from this, PAHs are continuously formed by incomplete combustion processes as anthropogenic products. Main sources of emission are domestic coal heating, coke production, open fires such as refuse burnings, forest fires and after-crop burning, but also vehicle exhaust and oil combustion. By these processes PAHs contaminate the environment *e.g.* air, water and soil from which they may enter vegetables and various other foodstuffs. Accordingly, PAHs are widely distributed throughout the environment.

It has been demonstrated by balancing the biological effect that for various emissions which permanently pollute the environment (such as vehicle exhaust, hard coal combustion, used motor oil) PAHs are the main contributors to the carcinogenic potential of these matrices compared to other classes of compounds present [7-12]. More recent investigations have also presented data on the ecotoxicity of some PAHs [for a review see ref. 13].

Due to their wide environmental distribution and to their carcinogenic potential, PAH require continuous analytical monitoring. As a further consequence, in some countries national recommendations and regulations have set limits for the emission and exposure to benzo[a]pyrene as typical representative of PAHs. There are presently well-justified tendencies to include some individual PAHs for the assessment of the carcinogenic potential of matrices and products as is the practice for polychlorinated furans and dioxins already.

More recently, the interest of environmental toxicologists has been focused on some classes of heterocyclic compounds and various PAH derivatives since potent carcinogens have been found among the thiaarenes (sulfur-containing polycyclic aromatic compounds) [14] and the azaarenes (nitrogen-containing polycyclic aromatic compounds) [15]. On the other hand, aromatic amines have been found to be potent bladder carcinogens and nitro-PAHs which occur in considerable concentrations in vehicle exhaust possess high mutagenic activities [16].

PAHs are not carcinogenic by themselves but require enzymatical activation to display biological [mutagenic and/or carcinogenic] effects. Cytochrom P450- dependent monooxygenases and hydrases are responsible for the activation converting PAHs into phenols (via epoxides) and trans-dihydrodiols. The latter ones are considered to function as proximate carcinogens which may be further activated to trans-dihydrodiol epoxides - the ultimate carcinogens of PAHs. The determination of these metabolites is of essential interest in biological monitoring e.g. for the risk assessment of people occupationally exposed to PAHs.

23.1 Availability of Reference Materials

A precondition for the determination of PAHs and their derivatives is the availability of pure reference materials for reasons of (i) identification and (ii) calibration.

Therefore, the Commission of the EC initiated a project in the frame of the BCR Programme in close collaboration with a number of specialist laboratories of the member states. The aim of this project was to provide PAH-reference materials with certified purity for those compounds for which demand could not be met from commercial sources. In total, 63 polycyclic aromatic compounds have been synthesized with a purity mostly of > 99.0% [17-19] selected on the base of (i) carcinogenic and toxicological aspects, (ii) non-availability from commercial suppliers, and (iii) environmental pollution considerations. These compounds are listed in Table 1. Their physical data have been published elsewhere [13,20,21].

Further to these pure compounds, a dried sewage sludge [22] has been certified with regard to its PAH content (pyrene, benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, indeno[1,2,3-cd]pyrene, benzo[b]naphtho[2,1-d]thiophene) by BCR and another material (coconut oil [23]) is in preparation for which the concentration of pyrene, benzo[a]pyrene, chrysene, benzo[k]fluoranthene, indeno[1,2,3-cd]pyrene and benzo[ghi]perylene will be certified.

Table 1 Polycyclic aromatic compounds available as reference material with a purity >99.0% [except those marked with*]

Compound	empirical formula	molecular weight	certified purity [%]
Parent PAH			
Fluoranthene	C ₁₆ H ₁₀	202	99.4
Pyrene	C ₁₆ H ₁₀	202	99.7
Benzo[ghi]fluoranthene	C ₁₈ H ₁₀	226	99.4
Benz[a]anthracene	C ₁₈ H ₁₂	228	99.7
Triphenylene	C ₁₈ H ₁₂	228	99.7
Chrysene	C ₁₈ H ₁₂	228	99.2
Benzo[c]phenanthrene	C ₁₈ H ₁₂	228	99.7
Benzo[a]fluoranthene	C ₂₀ H ₁₂	252	99.5
Benzo[b]fluoranthene	C ₂₀ H ₁₂	252	99.5
Benzo[j]fluoranthene	C ₂₀ H ₁₂	252	99.5
Benzo[k]fluoranthene	C ₂₀ H ₁₂	252	99.5
Benzo[a]pyrene	C ₂₀ H ₁₂	252	99.3
Benzo[e]pyrene	C ₂₀ H ₁₂	252	99.0
Anthanthrene	C ₂₂ H ₁₂	276	99.5
Benzo[ghi]perylene	C ₂₂ H ₁₂	276	99.0
Indeno[1,2,3-cd]fluoranthene	C ₂₂ H ₁₂	276	99.7
Indeno[1,2,3-cd]pyrene	C ₂₂ H ₁₂	276	99.0
Picene (benzo[a]chrysene)	C ₂₂ H ₁₄	278	99.8
Benzo[b]chrysene	C ₂₂ H ₁₄	278	99.5
Benzo[c]chrysene	C ₂₂ H ₁₄	278	99.5
Dibenz[a,c]anthracene	C ₂₂ H ₁₄	278	99.5
Dibenz[a,h]anthracene	C ₂₂ H ₁₄	278	99.0
Dibenz[a,j]anthracene	C ₂₂ H ₁₄	278	99.7
Coronene	C ₂₄ H ₁₂	300	99.8
Dibenzo[a,e]fluoranthene	C ₂₄ H ₁₄	302	99.8
Dibenzo[a,e]pyrene	C ₂₄ H ₁₄	302	99.6
Dibenzo[a,h]pyrene	C ₂₄ H ₁₄	302	99.1
Dibenzo[a,i]pyrene	C ₂₄ H ₁₄	302	99.6
Dibenzo[a,l]pyrene	C ₂₄ H ₁₄	302	99.6
Methyl-PAH			
1-Methyl-benz[a]anthracene	C ₁₉ H ₁₄	242	99.3
1-Methyl-chrysene	C ₁₉ H ₁₄	242	99.0
2-Methyl-chrysene	C ₁₉ H ₁₄	242	99.2
3-Methyl-chrysene	C ₁₉ H ₁₄	242	99.2
4-Methyl-chrysene	C ₁₉ H ₁₄	242	99.2
5-Methyl-chrysene	C ₁₉ H ₁₄	242	99.5
6-Methyl-chrysene	C ₁₉ H ₁₄	242	99.8
Thiaarenes			
Benzo[b]naphto[1,2-d] thiophene	C ₁₆ H ₁₀ S	234	99.7
Benzo[b]naphto[2,1-d] thiophene	C ₁₆ H ₁₀ S	234	99.5
Benzo[b]naphto[2,3-d] thiophene	C ₁₆ H ₁₀ S	234	99.4

(Table 1 continued)

Azaarenes			
Benz[a]acridine	$C_{17}H_{11}N$	229	99.7
Benz[c]acridine	$C_{17}H_{11}N$	229	99.8
10-Azabenz[a]pyrene	$C_{19}H_{11}N$	253	99.5
7H-Dibenzo[c,g]carbazole	$C_{20}H_{13}N$	267	99.6
Dibenz[a,c]acridine	$C_{21}H_{13}N$	279	99.8
Dibenz[a,h]acridine	$C_{21}H_{13}N$	279	99.8
Dibenz[a,i]acridine	$C_{21}H_{13}N$	279	99.8
Dibenz[a,j]acridine	$C_{21}H_{13}N$	279	99.8
Dibenz[c,h]acridine	$C_{21}H_{13}N$	279	99.3
Oxaarenes			
Dibenzo[b,d]furan	$C_{12}H_8O$	168	99.0
Benzo[b]naphto[1,2-d]furan	$C_{16}H_{10}O$	218	99.7
Benzo[b]naphto[2,1-d]furan	$C_{16}H_{10}O$	218	99.6
Nitro-PAH			
1-Nitronaphthalene	$C_{10}H_7NO_2$	173	99.6
2-Nitronaphthalene	$C_{10}H_7NO_2$	173	99.7
9-Nitroanthracene	$C_{14}H_9NO_2$	223	99.7
2-Nitro-7-methoxy-naphtho[2,1-d]furan	$C_{13}H_9O_4N$	243	99.8
1-Nitropyrene	$C_{16}H_9NO_2$	247	99.7
3-Nitrofluoranthene	$C_{16}H_9NO_2$	247	99.7
6-Nitrochrysene*	$C_{18}H_{11}NO_2$	273	98.9*
6-Nitrobenzo[a]pyrene	$C_{20}H_{11}NO_2$	297	99.8
PAH-ketones			
4-Cyclopenta[def]phenanthren-4-one	$C_{15}H_8O$	204	99.6
Benzo[a]fluorenone	$C_{17}H_{10}O$	230	99.8
6H-Benzo[cd]pyren-6-one*	$C_{19}H_{10}O$	254	98.9*
PAH-metabolites			
3-Hydroxybenzo[a]pyrene	$C_{20}H_{12}O$	268	99.4

Other materials have been prepared *e.g.* by the National Institute of Standards and Technology (NIST), (shale oil (SRM 1580), urban particulate matter (SRM 1648), urban dust (SRM 1649)) [24] and by the National Institute for Environmental Studies in Japan (vehicle exhaust particulates, NIES-CRM No. 8). All these materials can help to ensure the accuracy of the various analytical methods for the determination of PAHs and to improve the comparability of results.

23.2 Extraction of PAH

The initial step of PAH enrichment from environmental matter for their determination is extraction for which a great variety of solvents has been applied. The choice of solvent depends on which classes of compounds (*e.g.* PAH derivatives such as azaarenes, amines, ketones or PAH metabolites) are intended to be simultaneously extracted. The main problems arising from the extraction procedure are: (i) contamination of the sample, (ii) degradation of the sample, (iii) insufficient extraction and (iv) evaporation losses.

Sample contamination during the extraction can be avoided by carefully cleaning all glassware and by using freshly redistilled solvents to which air contact should be excluded or at least minimized.

Many PAHs are photosensitive and may undergo photooxidation. This holds especially for PAHs with anthracenoid partial structures which may be converted into endoperoxides. However, cyclopenta[cd]pyrene, anthanthrene and even benzo[a]pyrene have also been reported to be very sensitive and may be lost partially or completely during the cleanup procedure. Hence, exposure to UV light (sunlight) and to active oxygen (peroxides in ether, hydroperoxide) has to be carefully avoided.

The extractability of PAHs depends not only upon the solvent used for the extraction, but also on the matrix to be extracted. PAHs can readily be extracted from inorganic adsorbents but are difficult to extract quantitatively from carbonaceous materials such as carbon black and soot particles to which they are mainly adsorbed in the atmosphere. A great variety of solvents (cyclohexane, benzene, toluene, carbon disulphide, dichloromethane, chloroform, acetone, diethyl ether, methanol, ethanol, dioxane, tetrahydrofuran *etc.*) have been tested for the extraction of PAHs, and results of these investigations and, as a consequence, recommendations drawn from them are fairly controversial. In line with a method recommended by Schuetzle *et al.* [25], many laboratories, for instance, are still using dichloromethane or dichloromethane/methanol for PAH extraction. From Table 2 and 3, however, it becomes obvious that this solvent is less efficient than toluene, especially for the extraction of higher boiling PAH. This was also recently confirmed in an interlaboratory study within BCR carried out during the certification of the above-mentioned sewage sludge [22]. Only 64-67 % of the benzo[a]pyrene and indeno[1,2,3-cd]pyrene were extracted from the sample with dichloromethane.

It seems that ultrasonic extraction is superior to Soxhlet extraction. Since desorption, however, is temperature-dependent, hot extraction with solvent (e.g. refluxing with toluene) is recommended. A considerable improvement has been achieved by the Soxtec[®] principle by which the solid sample is extracted by boiling solvent prior to a regular short-term Soxhlet extraction. This method is very efficient and time-saving [27].

From the numerous investigations it may be concluded that extraction of PAHs with boiling toluene is the most appropriate method following the classical rule of chemists "*similia similibus solvuntur*".

Apart from solvent extraction, thermodesorption and sublimation techniques have been described which have mainly been applied for extracting PAHs from air particulates, often in direct combination with a detection system (capillary gas chromatography). Thermodesorption is almost quantitative for lower boiling PAHs, but less efficient for five-membered and higher PAH systems.

Occasionally, PAHs are difficult to extract from heterogeneous organic matrices (plant and animal tissues). In these cases alkaline saponification is required towards which most PAHs are stable, whereas acidic treatment often leads to artefact formation. Saponification results in homogenous phases from which PAHs may be readily extracted.

From aqueous matrices (sea, fresh, or waste water) PAHs can be directly extracted by distribution against toluene or other lipophilic (apolar) solvents. Solid phase extraction using Tenax, XAD, activated carbon or polyurethane is also widely used.

Table 2 Extraction of PAH from a filter loaded with Diesel exhaust, using toluene or dichloromethane (3 h at boiling point: 150 ml each) (ng) (from Grimmer *et al.*, 1982 [26]).

Extraction	Toluene			Dichloromethane		
	1st	2nd	1+2	1st	2nd	1+2
Fluoranthene	12131	246	12377	11500	149	11649
Pyrene	13990	423	14413	13263	139	13402
Benzo[b]naphtho[2,1-d]thiophene	250	2	252	235	20	255
Benzo[c]phenanthrene	2890	138	3028	2751	131	2882
Cyclopenta[cd]pyrene	148	28	176	71	14	85
Benz[a]anthracene	528	30	558	371	17	388
Chrysene/Triphenylene	2272	81	2353	2255	147	2402
Benzofluoranthenes[b+j+k]	2867	235	3102	2611	294	2905
Benzo[e]pyrene	1023	69	1092	1013	140	1153
Benzo[a]pyrene	115	22	137	20	5	25
Indeno[1,2,3-cd]pyrene	934	95	1029	539	137	676
Benzo[ghi]perylene	282	38	320	141	54	195
Coronene	594	103	697	218	91	309
Picene (1.520 ng added)	1261	231	1492	658	264	922

The use of supercritical fluids (e.g. CO₂, but also NH₃, N₂O, SF₆) has become a routine technique recently. Although more comparative studies are needed, it still appears that solvent extraction is more suitable for PAHs than supercritical fluid extraction (SFE). Especially higher molecular PAHs (benz[a]anthracene, benzo[a]pyrene) are less efficiently extracted from soil and sediment standard reference materials by SFE when compared to Soxtec^R-extraction [28].

Table 3 Extraction of PAHs from glass fibre filters, loaded with Diesel exhaust emission with several solvent. Yield after two extractions with boiling solvents compared with toluene (three times). Conditions see Table 2 (%) (from Grimmer *et al.*, 1982 [26]).

	Methanol	Acetone	Cyclo-hexane*	Dichloro-methane	Toluene	Cyclo-hexane**
Fluoranthene	96.7	99.4	97.2	99.4	99.9	97.7
Pyrene	98.0	99.7	98.3	99.6	100.0	98.5
Benzo[b]naphtho[2,1-d]thiophene	85.8	82.9	80.3	87.4	100.0	89.3
Benzo[c]phenanthrene	94.4	95.6	93.8	99.7	99.8	92.2
Cyclopenta[cd]pyrene	39.5	43.6	26.5	50.0	99.1	71.8
Benz[a]anthracene	83.4	76.8	85.5	72.2	99.0	59.1
Chrysene/Triphenylene	84.5	97.6	91.6	94.1	99.6	91.5
Benzo[fluoranthenes	82.2	82.6	80.3	89.7	98.9	62.6
[b+j+k]						
Benzo[e]pyrene	74.2	94.1	83.9	100.0	99.0	66.8
Benzo[a]pyrene	40.6	41.7	12.0	17.1	96.6	12.0
Indeno[1,2,3-cd]pyrene	22.8	40.5	42.6	61.9	97.2	33.6
Benzo[ghi]perylene	25.8	38.8	20.0	56.1	95.2	20.7
Coronene	8.5	15.4	18.8	40.6	94.4	14.4
Picene (1.520 ng = 100%)	39.8	25.6	80.3	60.9	99.5	52.4

* at boiling temperature

** ultrasound (3h) at room temperature

23.3 Enrichment and clean-up

Although time-consuming, a further enrichment of the PAH extract is indispensable in order to get reliable analytical results. Only from a highly purified PAH fraction can chromatograms be obtained which allow a correct quantitative evaluation and even then there remain some overlaps of insufficiently resolved PAH peaks in both GLC and HPLC.

The sample clean-up strategy has to envisage the separation of PAHs from more apolar [aliphatic hydrocarbons] and more polar compounds (such as ketones, phenols *etc.*). Partition between solvent pairs is an efficient first enrichment step. Several solvent systems have been proposed, *e.g.* methanol-water-cyclohexane [29], acetone-water-cyclohexane [30], cyclohexane-nitromethane [31], dimethylsulfoxide-pentane [32] or dimethylformamide-water-cyclohexane [33,34]. Using the system dimethylformamide-water cyclohexane (9:1:19; v/v/v) the various PAHs (phenanthrene - coronene) are removed with the cyclohexane phase. After addition of water a distribution system DMF-water-cyclohexane (1:1:2; v/v/v) leaves 92-98 % of the PAHs in the organic phase, whereas all hydrophilic constituents remain in the water phase.

Further to the above distribution systems, extraction of PAHs with caffeine-containing 90% formic acid has been described and integrated into a general method for the determination of PAHs in fats and oils [35,36]. This extraction procedure is based on the observation that PAHs form complexes with caffeine [37-39].

Distribution between solvent pairs in most instances results in PAH fractions which require further treatment prior to the final detection. A combination of chromatographic steps consisting of filtration through silica gel and separation on Sephadex LH 20 leads to very pure PAH fractions. Chromatography on Sephadex LH 20 using propanol-2 as elutant even allows separation of PAHs with 2-3 rings from those with more than 3 rings to which all toxicologically relevant individuals belong.

23.4 Thin-layer chromatography [TLC]

In the past, TLC has traditionally been considered to be a semiquantitative method for the determination of PAH in view of (i) the limited repeatability of spot size and (ii) the poor chromatographic resolution of individual PAHs. The method, however, has greatly benefited from the introduction of small and homogenous particles as adsorbents resulting in a significantly improved technique known by the term "high performance thin-layer chromatography (HPTLC)". Moreover, plates with pre-concentration zones also allow the determination of PAHs in comparatively crude samples.

TLC is widely used for the determination of PAHs in water samples [40,41] and has been accepted as a recommended method by the Association of Official Analytical Chemists (AOAC) [42] and the International Union of Pure and Applied Chemistry (IUPAC) [43,44].

A variety of materials has been used for TLC separation (silica gel, alumina, Florisil, cellulose, acetylated cellulose, Porapak *etc.*) using adsorption, partition (normal and reverse phase) and complexation TLC, the latter taking advantage of the formation of charge-transfer complexes of PAHs with electron acceptors such as caffeine.

The poor resolution of a great number of PAHs within a distance of about 10 cm has been overcome practically by means of a two-dimensional TLC technique.

The determination of PAHs can be performed *in situ* by scanning densitometry, UV spectrophotometry or spectrofluorometry of the separated spots or their colour reaction products. Detection limits for benzo[a]pyrene of about 1 ng and less with standard deviations of about 10 % have been reported using *in situ* transmission or reflectance measurements by various authors. More sophisticated techniques such as low temperature fluorescence and phosphorescence have been successfully applied for a specific and quantitative detection of PAHs after scraping off the PAH-containing zones and solvent extraction of the PAHs. Recoveries of 70-100 % for the extraction have been reported.

In order to obtain reproducible results standardization of the relative humidity (water content) of the plates and a equilibration of the solvent system in the separation chamber are required. More analytical details of TLC of PAHs may be found in the very comprehensive review written by Daisey [45].

23.5 High-Performance Liquid Chromatography [HPLC]

High-performance liquid chromatography (HPLC) and gas-liquid chromatography (GC) are the preferred methods for the analytical separation of PAH fractions into individual compounds. Both techniques have their specific advantages and disadvantages. Using HPLC, the material is not decomposed by the separation conditions nor by the detector system (as it is in GC) so that the same sample can be further analyzed by additional methods. Even higher boiling PAHs may be determined because volatility of the sample is not the limiting factor in HPLC. The poor resolution of HPLC initially reported for individual PAHs in comparison to GC has been overcome by the availability of a large number of different separation phases and the introduction of small diameter capillary columns) as well as by temperature programming [46,47]. In normal phase HPLC a variety of chemically bonded polar phases (nitro-, amino-, nitro-) are used whereas monomeric and polymeric C_{18} -phases are predominantly applied in reverse phase HPLC. More recently, two materials have been introduced which are particularly recommended for the separation of PAH isomers: (2-nitrophenyl)ethyl-silyl- and (2-(1-pyrenyl)ethyl)silyl silica packing (commercially available as Cosmosil NPE and Cosmosil PYR). The latter has successfully been applied in the separation of the extremely high boiling point fullerenes (C_{60} and C_{70}) [48, 49] using normal-phase HPLC with toluene or toluene/hexane (1:3) as mobile phase.

More common for the separation of PAHs is the reverse phase mode for which a large variety of packing materials are available (e.g. octyl-, dodecyl-, octadecyl-, triacontyl-) which allow a complete separation of most of the common PAHs using methanol/water gradients. Data on column selectivity including Partisil 5-ODS, MicroPak MCH-10, Nucleosil 10 C_{18} , Zorbax ODS, LiChrosorb RP-18, MicroPak CH-10 and Vydac 201TP have been presented by Wise *et al.* [50]. For instance, the pairs benz[a]anthracene/chrysene and indeno[1,2,3-cd]pyrene/benzo[c]chrysene are not resolved when Zorbax ODS or LiChrosorb RP-18 are applied, whereas Vydac 201TP most efficiently separates these PAHs. Similarly to the case in the field of GC, the broad variety of columns possessing different separation specificities allow the resolution of practically all relevant PAHs. Table 4 presents some retention times of PAHs and some of the methyl derivatives recorded with normal and reverse phase HPLC [50-52].

Multi-dimensional HPLC consisting of normal-phase HPLC to primarily isolate PAH isomeric groups and subsequent reverse-phase HPLC has been applied for the separation of PAH isomers with molecular weights of 278 and 302 from 4 standard reference materials (coal tar, two samples of air particulate matter, marine sediment) amongst which potent carcinogens are found which have not regularly been analyzed in environmental samples so far [53]. In this study, a baseline separation of six isomers with molecular weights of 278 (dibenz[a,c]anthracene, dibenz[a,j]anthracene, pentaphene, dibenz[a,h]anthracene, benzo[b]chrysene and picene using dibenz[a,h]anthracene- d_{14} as internal standard) and nine isomeric PAHs with a molecular weight of 302 (naphtho[2,3-e]pyrene, dibenzo[a,e]pyrene, naphtho[1,2-k]fluoranthene, dibenzo[b,k]fluoranthene, naphtho[2,3-b]fluoranthene, naphtho[2,3-k]fluoranthene, dibenzo[a,i]pyrene, naphtho[2,3-a]pyrene, and dibenzo[a,h]pyrene using dibenzo[a,i]pyrene- d_{14} as internal standard) was achieved.

Wise *et al.* [54] have also measured the HPLC retention data for a number of sulfur-heterologous PAHs (Table 5).

Table 4 HPLC retention times for polycyclic aromatic hydrocarbons [log I][taken from refs [50-52]

Compound	Normal-phase NH ₂ column ^a	Reverse-phase C ₁₈	
		Polymeric ^b	Monomeric ^c
Naphthalene	2.00	2.00	2.00
Acenaphthene	2.10	2.64	2.51
Acenaphthylene	2.59	2.26	---
Fluorene	2.55	2.70	2.75
1-Methylfluorene	2.64	3.15 ^d	3.33
2-Methylfluorene	2.59	3.24 ^d	3.42
4-Methylfluorene	---	3.10 ^d	3.34
Anthracene	2.94	3.20	3.14
1-Methylanthracene	---	3.41 ^d	3.55
2-Methylanthracene	3.01	3.71 ^d	3.69
9-Methylanthracene	3.02	3.41 ^d	3.53
Phenanthrene	3.00	3.00	3.00
1-Methylphenanthrene	3.02	3.39 ^d	3.51
2-Methylphenanthrene	3.00	3.71 ^d	3.71
3-Methylphenanthrene	3.12	3.29 ^d	3.46
4-Methylphenanthrene	---	3.26 ^d	3.40
9-Methylphenanthrene	3.02	3.34 ^d	3.51
Benzo[a]fluorene	3.51	3.79 ^d	3.76
Benzo[b]fluorene	3.54	3.82 ^d	3.78
Benzo[c]fluorene	---	3.49 ^d	3.64
4,5-Methylenepheneanthrene	3.10	3.16	3.35
Pyrene	3.37	3.58 ^d	3.65
1-Methylpyrene	3.46	3.98 ^d	4.15
2-Methylpyrene	---	4.05 ^d	4.19
Fluoranthene	3.51	3.37	3.43
Benzo[c]phenanthrene	3.64	3.63	3.92
2-Methylbenzo[c]phenanthrene	---	3.83	4.23
3-Methylbenzo[c]phenanthrene	---	4.04	4.39
4-Methylbenzo[c]phenanthrene	---	4.01	4.38
5-Methylbenzo[c]phenanthrene	---	3.97	4.36
6-Methylbenzo[c]phenanthrene	---	3.94	4.38
Benz[a]anthracene	4.00	4.00	4.00
1-Methylbenz[a]anthracene	3.90	4.14	4.38
2-Methylbenz[a]anthracene	---	4.09	4.40
3-Methylbenz[a]anthracene	---	4.39	4.53
4-Methylbenz[a]anthracene	---	4.33 ^d	4.46
5-Methylbenz[a]anthracene	4.04	4.28	4.48
6-Methylbenz[a]anthracene	4.03	4.10	4.39

(Table 4 continued)

7-Methylbenz[a]anthracene	---	4.14	4.35
8-Methylbenz[a]anthracene	4.03	4.19	4.39
9-Methylbenz[a]anthracene	4.08	4.39	4.53
10-Methylbenz[a]anthracene	---	4.24	4.47
11-Methylbenz[a]anthracene	3.91	4.13	4.41
12-Methylbenz[a]anthracene	---	4.10	4.35
7,12 Dimethylbenz[a]anthracene	3.90	4.19 ^d	4.83
Chrysene	4.01	4.10	3.99
1-Methylchrysene	4.07	4.43	4.46
2-Methylchrysene	4.08	4.52	4.52
3-Methylchrysene	4.12	4.29	4.42
4-Methylchrysene	3.95	4.18	4.35
5-Methylchrysene	3.94	4.14	4.35
6-Methylchrysene	4.10	4.14	4.36
Triphenylene	4.07	3.73 ^d	3.83
Naphthacene	3.95	4.51 ^d	---
Cyclopenta[cd]pyrene	---	3.97 ^d	4.03
Benzo[ghi]fluoranthene	3.84	3.95 ^d	4.07
3-Methylcholanthrene	4.31	4.78	5.23
Benzo[b]fluoranthene	4.48	4.29	4.46
Benzo[j]fluoranthene	4.56	4.24	4.37
Benzo[k]fluoranthene	4.45	4.42	4.52
Benzo[a]pyrene	4.38	4.53	4.68
Benzo[e]pyrene	4.46	4.28	4.48
Perylene	4.61	4.33	4.50
Benzo[ghi]perylene	4.83	4.73	5.16
Anthanthrene	4.80	4.93	5.38
Indeno[1,2,3-cd]pyrene	4.90	4.83	5.13
Dibenz[a,c]anthracene	4.93	4.40 ^d	4.82
Dibenz[a,j]anthracene	---	4.51 ^d	4.82
Dibenz[a,h]anthracene	4.94	4.72 ^d	4.85
Dibenz[a,e]fluoranthene	---	4.93 ^d	---
Benzo[b]chrysene	5.00	5.00	5.00
Picene	5.03	5.10	5.31
Biphenyl	2.16	2.37	2.38
o-Terphenyl	2.50	2.97 ^d	3.31
m-Terphenyl	3.12	3.16 ^d	3.56
p-Terphenyl	3.28	3.74 ^d	3.75
m-Quaterphenyl	4.06	3.81 ^d	4.49
p-Quaterphenyl	4.50	5.04 ^d	5.48
m-Quinquephenyl	5.00	4.25 ^d	5.29

^aμBondapak NH₂ column, n-hexane as mobile phase; silica and alumina/n-pentane.

^bVydac 201TP column, 85% acetonitrile in water as mobile phase except for PAH with log I < 3.00.

^cZorbax ODS column, 80% acetonitrile in water as mobile phase.

^dLog I determined on a Vydac 201TP column from different lot.

Table 5 HPLC retention data for various thiaarenes of molecular weight 234, 258 and 284* [taken from ref. 53]

Compound	Normal-phase**	Reversed-phase***	
		I	II
Benzo[b]naphtho[1,2-d]thiophene	3.65	3.79	4.02
Benzo[b]naphtho[2,3-d]thiophene	3.78	4.05	4.05
Benzo[b]naphtho[2,1-d]thiophene	3.59	4.20	4.24
Anthra[2,3-b]thiophene	4.19	3.41	2.97
Phenanthro[3,4-b]thiophene	3.89	3.57	3.70
Phenanthro[2,3-b]thiophene	4.26	3.65	3.70
Phenanthro[3,2-b]thiophene	4.24	3.67	3.67
Phenanthro[9,10-b]thiophene	3.89	3.77	3.86
Anthra[2,1-b]thiophene	4.14	3.78	3.74
Phenanthro[4,3-b]thiophene	3.94	3.79	3.86
Phenanthro[2,1-b]thiophene	4.13	3.80	3.71
Anthra[1,2-b]thiophene	3.94	3.99	3.93
Phenanthro[1,2-b]thiophene	3.98	4.08	3.93
Triphenyleno[1,12-bcd]thiophene	4.09	4.30	4.61
Chyseno[4,5-bcd]thiophene	4.04	4.51	4.77
Benzo[2,3]phenanthro[4,5-bcd]thiophene	3.83	4.65	4.98
Benzo[1,2]phenanthro[4,3-bc]thiophene	---	4.16	4.81
Pyreno[4,5-b]thiophene	4.32	4.31	4.55
Benzo[1,2]phenaleno[3,4-bc]thiophene	---	4.35	4.62
Pyreno[2,1-b]thiophene	4.55	4.35	4.44
Benzo[4,5]phenaleno[9,1-bc]thiophene	---	4.36	4.51
Benzo[4,5]phenaleno[1,9-bc]thiophene	---	4.37	4.48
Pyreno[1,2-b]thiophene	4.32	4.45	4.62
Benzo[b]phenanthro[4,3-d]thiophene	4.00	4.18	4.80
Benzo[b]phenanthro[3,2-b]thiophene	4.91	4.52	4.84
Benzo[b]phenanthro[9,10-b]thiophene	4.49	4.68	5.17
Benzo[b]phenanthro[2,3-b]thiophene	4.81	4.74	4.91
Benzo[b]phenanthro[3,4-b]thiophene	4.49	4.81	5.21
Dinaphtho[2,3-b:2',3'-d]thiophene	4.77	4.84	5.12
Dinaphtho[1,2-b:1',2'-d]thiophene	4.33	4.95	5.27
Anthra[1,2-b]benzo[d]thiophene	4.52	>5	5.25
Dinaphtho[1,2-b:2',1'-d]thiophene	4.40	>5	5.51
Benzo[b]phenanthro[2,1-d]thiophene	4.58	>5	5.32
Dinaphtho[1,2-b:2',3'-d]thiophene	4.55	>5	5.38
Triphenyleno[2,1-b]thiophene	4.81	4.21	4.53
Triphenyleno[2,3-b]thiophene	4.16	4.30	4.61
Triphenyleno[1,2-b]thiophene	4.98	4.40	4.68

* Retention data given as log I related to log I of benzene (1.00), naphthalene (2.00),

phenanthrene (3.00), benz[a]anthracene (4.00) and benzo[b]chrysene (5.00) (from Wise *et al.*, 1980).

** μ Bondapak NH₂ column, n-hexane as the mobile phase.

*** I=Vydac 201TP 5 μ m and II=Zorbax ODS column, 85/15 (v/v) acetonitrile/water as the mobile phase.

Size exclusion or gel permeation chromatography which is regularly used for the clean-up of PAH fractions has been adapted to HPLC [55,56] but rarely used routinely.

More recently the efficiency of HPLC separation has been considerably increased by miniaturizing the column dimension, the introduction of capillary techniques (microbore, microcapillary and open-tubular microcapillary columns) and the application of smaller size particle packages. The characteristics of conventional and miniaturized HPLC columns are summarized in Table 6 [data taken from ref. 57].

Table 6 Characteristics of conventional and miniaturized HPLC columns [data taken from ref. [57]].

Column Type	Typical Dimensions		Flow-Rate	Sample Capacity	
	I.D.	Length			
Conventional Column	4.0-4.6mm	10 - 25 cm	1 mL.min ⁻¹	10	- 100µg
Small-Bore Packed Column	0.2-1 mm	1 - 10 m	1-20 µL.min ⁻¹	1	- 10µg
Packed Capillary Column	40 -80 µm	1 - 100 m	0.5-2µL.min ⁻¹	100 ng	- 1µg
Open-Tubular Capillary	15 -50 µm	1 - 100 m	< 1 µL.min ⁻¹	<100 ng	

High resolution (column plate counts of > 100.000) have been described for capillary columns at the price of long and occasionally very long (up to 20 hrs) elution times [58,59].

UV and fluorescence are commonly used for detection in HPLC. UV detection may be performed at a fixed or multiple wavelength(s) to get optimum responses for various PAHs. Stop and flow scanning and rapid scanning photodiode array on-line systems allow complete spectra to be recorded giving detailed information on the structure of the PAHs separated. For fluorescence detection, appropriate excitation and emission wavelengths have to be selected which are available for a great number of PAHs and related compounds from spectral data published in the literature [13,20,21].

The main disadvantage of HPLC detection is that response factors have to be determined prior to the measurement which, in contrast to FI-detection in GC, vary from one compound to another. Precondition for this calibration is the availability of the compounds in weighable amounts. Although unproblematic for most of the common PAHs it remains critical for minor components such as alkylated PAHs and PAH metabolites. According to the Lambert-Beer law responses are linear over several orders of magnitude. The above detector systems are very sensitive and allow the detection of 10 - 100 pg in case of fluorescence and about 50- 1000 ng in case of UV detection.

The application of more sophisticated detector systems such as electrochemiluminescence and photoionization devices have been described by which some 10-20 pg may be recorded. However, they are not routinely used. HPLC may also be coupled to mass spectrometry since the initial problems of this combination have been overcome so that detailed information on the molecular structure may be obtained by this technique which has been a feature of GC-MS for a long time.

23.6 Gas-liquid chromatography [GC]

Alternatively or supplementary to HPLC, gas-liquid chromatography is commonly used for the determination of PAHs. The initially applied packed columns (2m up to 50 m in length) have now been replaced by capillaries (glass and fused silica; coated or chemically bonded) (10-50 m, exceptionally up to 100 m in length) with a high resolution power for PAHs and related compounds. A great number of different, mainly apolar phases have been applied such as methylpolysiloxanes, 5 % phenylmethylpolysiloxanes, 1 % vinyl/5 % phenyl/94 % methylpolysiloxane and others were used for the separation of certain PAH which were not resolved with apolar phases. A selection of commonly used phases are listed in Table 7.

Table 7 Phases used for the GC determination of PAH

Phase	Trade name	Polarity
Methylpolysiloxane	BP-1, CP-Sil 5, DB-1, GB-1, HP-1, HP-101, OV-1, OV-101, RSL-150, Rtx-1, SA-1, SE 30, SPB-1, SP-2100, Ultra-1, 007-1	apolar
5%phenyl/95%methylpolysiloxane	BP-5, CB-5, BPX-5, CP-Sil 8, DB-5, DM-5ms, GC-5, HP-2, HP-5, OV-5, OV-73, PTE-5, RSL-200, Rtx-5, SA-5, SE-52, SPB-5, Ultra-2, XT1-5, 007-2	
1%vinyl/5%phenyl/94%methylpolysiloxane	SE 54	
14%phenyl/86%methylpolysiloxane	BP-10, CP-Sil 13, DB-624, OV 17, SA-624, 007-624	
7%phenyl/7%cyanopropyl/86%methylpolysiloxane or 14%cyanopropyl/86% methylpolysiloxane	BP-10, CB-1701, CP-Sil 19CB, DB-1701, OV-1701, Rtx-1701, SA-1701, SPB-7, 007-1701	
50%cyanopropyl/50%methylpolysiloxane	BP-225, CB-225, CP-Sil 43CB, DB-225, OV-225, HP-225, Rtx-225, RSL-500, 007-225, Silar 5 CP	polar

Because of the limited stability of the phases even in case of chemically-bonded ones, which lead to bleeding and accordingly to high background noise in detection working temperatures may rarely exceed 320 °C.

Though high-temperature phases have been developed [62] and compounds with molecular weights above 500 have been analyzed with short columns [63], routine analysis of PAHs finds its realistic end in GC with PAHs possessing molecular weights below about 350. For the determination of higher PAHs, at least at present, HPLC is the recommended technique.

Even when highly efficient columns are applied, there remain separation problems for some PAH couples such as chrysene/triphenylene or benzo[b]-, [j]- and [k]fluoranthene. They, however, can be separated by using liquid crystal phases such as N, N'-bis-(p-alkoxy-benzylidene)- α,α' -bis-p-toluidine (with alkoxy residues: methoxy-(designated as BMBT); n-propoxy- (BPrBT); n-butoxy- (BBBT); n-hexyloxy (BHxBT) or phenoxy- (BPhBT).

Retention times for a variety of PAH, thiaarenes and azaarenes are presented in Tables 8-10).

Table 8 Relative GC retention times (RRT) of various PAH (related to naphthalene (=200.00), phenanthrene (=300.00), chrysene (=400.00) and picene (=500.00) for SE-52-coated fused silica columns (from Vassilaros *et al.* [64]).

Compound	Molecular weight	Retention time
Naphthalene	128	200.00
2-Methylnaphthalene	142	220.22
1-Methylnaphthalene	142	223.01
Biphenyl	154	236.44
2,6/2,7-Dimethylnaphthalene	156	240.28
1,7-Dimethylnaphthalene	156	242.77
1,3/1,6-Dimethylnaphthalene	156	243.30
1,4/2,3-Dimethylnaphthalene	156	246.03
1,5-Dimethylnaphthalene	156	246.92
Acenaphthylene	152	246.92
1,2-Dimethylnaphthalene	156	248.50
1,8-Dimethylnaphthalene	156	252.00
Acenaphthene	154	253.14
3-/4-Methylbiphenyl	168	256.69
1-Methylacenaphthylene	166	266.57
Fluorene	166	269.73
9-Methylfluorene	180	273.79
2-Methylfluorene	180	288.42
1-Methylfluorene	180	289.20
Phenanthrene	178	300.00
Anthracene	178	301.08
3-Methylphenanthrene	192	319.19
2-Methylphenanthrene	192	319.93
4H-Cyclopenta[def]phenanthrene	190	321.77
4-/9-Methylphenanthrene	192	322.81
1-Methylphenanthrene	192	323.64
2-Phenylnaphthalene	204	332.64
Fluoranthene	202	344.51
Acephenanthrylene	202	347.67
Pyrene	202	351.51
Benzo[a]fluorene	216	366.72
Benzo[b]fluorene	216	369.40
1-Methylpyrene	216	373.45
Benzo[ghi]fluoranthene	226	389.92
Benzo[c]phenanthrene	228	391.24

(Table 8 continued)

Cyclopenta[cd]pyrene	226	396.55
Benz[a]anthracene	228	398.76
Chrysene	228	400.00
Triphenylene	228	400.00
3-Methylchrysene	242	418.02
2-Methylchrysene	242	419.66
5-Methylchrysene	242	420.20
4-/6-Methylchrysene	242	421.25
1-Methylchrysene	242	422.99
Benzo[j]/[b]fluoranthene	252	443.13
Benzo[k]fluoranthene	252	444.02
Benzo[e]pyrene	252	452.29
Benzo[a]pyrene	252	454.02
Perylene	252	457.17
Indeno[1,2,3-cd]pyrene	276	493.24
Dibenz[a,h]anthracene	278	496.20
Dibenz[a,c]anthracene	278	497.09
Benzo[b]chrysene	278	498.90
Picene	278	500.00
Benzo[ghi]perylene	276	500.29
Anthanthrene	276	504.10

Table 9 Relative GC retention times (RRT) of various thiaarenes (related to naphthalene (=200.00), phenanthrene (=300.00), chrysene (=400.00) and picene (=500.00) for SE-52-coated fused-silica columns (from Vassilaros *et al.* [64]).

Compound	Molecular weight	Retention time
Benzo[b]thiophene	134	201.57
7-Methylbenzo[b]thiophene	148	219.16
2-Methylbenzo[b]thiophene	148	220.76
5-Methylbenzo[b]thiophene	148	222.09
6-Methylbenzo[b]thiophene	148	222.11
3-Methylbenzo[b]thiophene	148	223.08
4-Methylbenzo[b]thiophene	148	223.15
5-Ethylbenzo[b]thiophene	162	236.14
3,5-Dimethylbenzo[b]thiophene	162	243.56
1,2,3,4,4a,4b-Hexahydrodibenzothiophene	190	271.69
Naphtho[1,2-b]thiophene	184	295.80
Dibenzothiophene	184	296.01
Naphto[2,1-b]thiophene	184	300.00
Naphto[2,3-b]thiophene	184	304.47
5-Methylnaphtho[2,1-b]thiophene	198	306.53
2-Methylnaphtho[2,1-b]thiophene	198	311.77
4-Methyldibenzothiophene	198	312.72
8-Methylnaphtho[2,1-b]thiophene	198	315.61
2-Methyldibenzothiophene	198	316.19
3-Methyldibenzothiophene	198	316.32
4-Methylnaphtho[1,2-b]thiophene	198	317.19

(Table 9 continued)

4-Methylnaphtho[2,1-b]thiophene	198	318.12
6-Methylnaphtho[1,2-b]thiophene	198	319.55
1-Methyldibenzothiophene	198	319.69
8-Methylnaphtho[2,1-b]thiophene	198	319.86
7-Methylnaphtho[2,1-b]thiophene	198	320.26
6-Methylnaphtho[2,1-b]thiophene	198	323.57
1-Methylnaphtho[2,1-b]thiophene	198	323.58
9-Methylnaphtho[2,1-b]thiophene	198	325.25
3-Ethyldibenzothiophene	212	328.34
4,6-Dimethyldibenzothiophene	212	329.17
2,6-Dimethyldibenzothiophene	212	332.42
2-Ethyldibenzothiophene	212	332.65
3,6-Dimethyldibenzothiophene	212	332.88
2,8-Dimethyldibenzothiophene	212	335.90
3,7-Dimethyldibenzothiophene	212	336.02
3,8-Dimethyldibenzothiophene	212	336.09
1,7-Dimethyldibenzothiophene	212	339.36
Phenanthro[4,5-bcd]thiophene	208	348.75
Phenaleno[6,7-bc]thiophene	208	353.45
Benzo[b]naphtho[2,1-d]thiophene	234	389.37
Benzo[b]naphtho[1,2-d]thiophene	234	392.92
Phenanthro[9,10-b]thiophene	234	394.96
Phenanthro[4,3-b]thiophene	234	395.03
Anthra[1,2-b]thiophene	234	395.39
Benzo[b]naphtho[2,3-d]thiophene	234	395.97
Phenanthro[1,2-b]thiophene	234	396.01
Phenanthro[3,4-b]thiophene	234	396.43
Anthra[2,1-b]thiophene	234	399.31
Phenanthro[2,1-b]thiophene	234	400.59
Phenanthro[3,2-b]thiophene	234	401.89
Phenanthro[2,3-b]thiophene	234	402.19
1-Methylbenzo[b]naphtho[1,2-d]thiophene	248	402.59
11-Methylbenzo[b]naphtho[1,2-d]thiophene	248	404.15
10-Methylbenzo[b]naphtho[2,1-d]thiophene	248	404.28
3-Methylbenzo[b]naphtho[2,1-d]thiophene	248	407.55
Anthra[2,3-b]thiophene	234	407.57
2-Methylbenzo[b]naphtho[2,1-d]thiophene	248	407.63
8-Methylbenzo[b]naphtho[2,1-d]thiophene	248	407.69
9-Methylbenzo[b]naphtho[2,1-d]thiophene	248	407.93
2-Methylbenzo[b]naphtho[1,2-d]thiophene	248	408.00
8-Methylbenzo[b]naphtho[1,2-d]thiophene	248	409.04
10-Methylbenzo[b]naphtho[1,2-d]thiophene	248	409.04
6-Methylbenzo[b]naphtho[1,2-d]thiophene	248	409.48
5-Methylbenzo[b]naphtho[2,1-d]thiophene	248	410.58
3-Methylbenzo[b]naphtho[1,2-d]thiophene	248	411.48
4-Methylbenzo[b]naphtho[2,3-d]thiophene	248	411.60
4-Methylbenzo[b]naphtho[2,1-d]thiophene	248	411.65
6-Methylbenzo[b]naphtho[2,1-d]thiophene	248	411.71
9-Methylbenzo[b]naphtho[1,2-d]thiophene	248	411.81
7-Methylbenzo[b]naphtho[2,1-d]thiophene	248	412.08
10-Methylbenzo[b]naphtho[2,3-d]thiophene	248	414.26
9-Methylbenzo[b]naphtho[2,3-d]thiophene	248	414.62
1-Methylbenzo[b]naphtho[2,1-d]thiophene	248	414.62
8-Methylbenzo[b]naphtho[2,3-d]thiophene	248	414.68

(Table 9 continued)

2-Methylbenzo[b]naphtho[2,3-d]thiophene	248	414.69
6-Methylbenzo[b]naphtho[2,3-d]thiophene	248	415.02
3-Methylbenzo[b]naphtho[2,3-d]thiophene	248	415.11
4-Methylbenzo[b]naphtho[1,2-d]thiophene	248	415.41
1-Methylbenzo[b]naphtho[2,3-d]thiophene	248	415.54
7-Methylbenzo[b]naphtho[2,3-d]thiophene	248	417.07
3-Methylphenanthro[9,10-d]thiophene	248	417.70
1-Methylanthra[2,1-b]thiophene	248	418.22
10-Methylphenanthro[2,1-b]thiophene	248	422.14
11-Methylbenzo[b]naphtho[2,3-d]thiophene	248	422.85
3-Methylphenanthro[2,1-b]thiophene	248	423.48
2-(2'-Naphthyl)benzo[b]thiophene	260	430.65
Benzo[2,3]phenanthro[4,5-bcd]thiophene	258	443.29
Pyreno[4,5-b]thiophene	258	446.51
Benzo[1,2]phenaleno[3,4-bc]thiophene	258	447.66
Triphenyleno[1,12-bcd]thiophene	258	448.45
Pyreno[2,1-b]thiophene	258	449.30
Chryseno[4,5-bcd]thiophene	258	450.62
Pyreno[2,1-b]thiophene	258	455.01
Benzo[4,5]phenaleno[1,9-bc]thiophene	258	455.99
Benzo[4,5]phenaleno[9,1-bc]thiophene	258	457.30
Benzo[b]phenanthro[4,3-d]thiophene	284	470.47
Dinaphtho[2,1-b:1',2'-d]thiophene	284	472.62
Dinaphtho[1,2-b:2',1'-d]thiophene	284	482.60
Benzo[1,2]phenaleno[4,3-bc]thiophene	284	482.99
Dinaphtho[1,2-b:1',2'-d]thiophene	284	486.58
Benzo[b]phenanthro[9,10-d]thiophene	284	487.32
Benzo[b]phenanthro[3,4-d]thiophene	284	487.76
Anthra[1,2-b]benzo[d]thiophene	284	488.45
Benzo[b]phenanthro[2,1-d]thiophene	284	488.89
Dinaphtho[1,2-b:2',3'-d]thiophene	284	489.14
9,13-H-Triphenyleno[2,3-b]thiophene	286	489.81
Benzo[b]phenanthro[3,2-d]thiophene	284	491.02
Benzo[b]phenanthro[1,2-d]thiophene	284	492.31
Benzo[b]phenanthro[2,3-d]thiophene	284	493.31
Triphenyleno[2,1-b]thiophene	284	493.90
Triphenyleno[1,2-b]thiophene	284	494.41
Dinaphtho[2,3-b:2',3'-d]thiophene	284	495.17
Triphenyleno[2,3-b]thiophene	284	500.00
13-Methylbenzo[b]phenanthro[3,2-d]thiophene	298	511.19

Table 10 Relative GC retention times (RRT) of various azaarenes (related to naphthalene (=200.00), phenanthrene (=300.00), chrysene (=400.00) and picene (=500.00) for SE-52-coated fused-silica columns (from Vassilaros *et al.* [64]).

Compound	Molecular weight	Retention time
Quinoline	129	210.26
Isoquinoline	129	214.14
1-Methylindole	131	216.90
Indole	117	222.66
7-Azaindole	118	223.70
2-Methylquinoline	143	224.13
8-Methylquinoline	143	225.18
1-Methylisoquinoline	143	229.21
7-Methylquinoline	143	231.37
3-Methylquinoline	143	232.47
7-Methylindole	131	235.49
4-Methylquinoline	143	235.77
3-Methylindole	131	239.20
2-Methylindole	131	240.10
2,7-Dimethylquinoline	157	244.04
2,6-Dimethylquinoline	157	244.19
1,2-Dimethylindole	145	244.42
2,4-Dimethylquinoline	157	247.96
2,5-Dimethylindole	145	256.65
2,3-Dimethylindole	145	257.32
2,3,5-Trimethylindole	159	273.61
4-Azafluorene	167	279.85
Phenazine	180	294.37
Benzo[h]quinoline	179	301.94
Acridine	179	304.04
Acridan (9,10-dihydroacridine)	181	304.11
Benzo[f]quinoline	179	307.94
Phenanthridine	179	307.94
Carbazole	167	311.71
3-Methylbenzo[f]quinoline	193	320.26
2-Methylbenzo[f]quinoline	193	320.50
2-Methylacridine	193	324.34
1-Methylcarbazole	181	324.45
3-Methylcarbazole	181	328.81
2-Methylcarbazole	181	329.61
9-Methylacridine	193	331.15
4-Methylcarbazole	181	331.88
6-Phenylquinoline	205	340.84
1,4-Dimethylcarbazole	195	343.16
2-Phenylindole	193	346.18
1,2-Dimethylcarbazole	195	347.31
2-Azafluoranthene	203	347.39
1-Azafluoranthene	203	348.17
1,3-Dimethylcarbazole	195	348.45
7-Azafluoranthene	203	350.50
1-Azapyrene	203	357.73

(Table 10 continued)

4-Azapyrene	203	357.94
2-Azapyrene	203	362.43
Benzo[def]carbazole	191	363.92
9-Phenylcarbazole	243	381.51
Benz[c]acridine	229	392.60
Benz[a]acridine	229	398.65
1-Azabenz[a]anthracene	229	400.00
4-Azachrysene	229	401.16
Benzo[a]carbazole	217	402.22
1-Azachrysene	229	407.18
Benzo[b]carbazole	217	409.63
2-Azachrysene	229	411.49
Benzo[c]carbazole	217	411.89
7,9-Dimethylbenz[c]acridine	257	438.32
5,7-Dimethylbenz[a]acridine	257	438.38
7,10-Dimethylbenz[a]acridine	257	439.46
10-Azabenz[a]pyrene	253	455.40
Dibenz[a,h]acridine	279	488.55
Dibenzo[a,i]carbazole	267	490.57
Dibenz[a,j]acridine	279	490.66
Dibenzo[a,g]carbazole	267	502.30
Dibenzo[c,g]carbazole	267	502.92

Depending upon whether isothermal or temperature-programmed conditions are chosen, split or splitless injection is recommended. In case of split injection (split ratio 1:10 - 1:100) which is applied in case of isothermal GC *e.g.* for the exact measurement of relative retention times, equivalent chain lengths or other GC retention indices, sensitivity problems may arise from the limitation of the sample size especially for higher boiling components. In both, splitless (split/splitless) and cold on-column injection, the injection temperature depends on the boiling point of the solvent in which the sample is dissolved. In case of on-column injection peak broadening and accordingly a reduced peak resolution is observed for higher boiling PAHs when a low boiling solvent is used. Nevertheless, standard deviations of the peak areas were found to be markedly smaller in case of cold on-column injection than with split or splitless injection [65]. To avoid pre-evaporation effects during the injection, which may lead to discrimination of the sample composition, special devices are commercially available. Practically speaking, it is recommended to firstly introduce 1-2 μl of solvent into the injection syringe, then 1 μl of air, followed by about 1 μl of the sample solution and finally another 1-2 μl of air. During the injection the needle should be empty and reach the inlet temperature prior to the shock-like injection of the sample. During this procedure the 1-2 μl of the solvent act as sample driver. For measuring highly volatile PAH the purge-and-trap technique has recently become common.

For the detection of PAHs separated by gas-chromatography a flame ionization detector (FID) is most commonly used. This detector gives linear responses over at least five orders of magnitude and possesses a high sensitivity with limits of detection of *e.g.* 100 pg phenanthrene, 500 pg benzo[a]pyrene and about 1 ng benzo[ghi]perylene. An advantage of this detector system, when compared to UV or fluorescence detectors used

in HPLC, is its carbon-dependent response which is almost identical for all PAHs. Thus, response factors for individual PAH may be calculated from their formula and do not have to be determined experimentally. This advantage is lost when electron capture detectors (ECD) are used since here response factors depend on the PAH structure [66,67]. On the other hand, the sensitivity is up to a factor of 300 higher than in case of FID and reaches the femtogram range as may be seen from Table 11.

Table 11 Ratio of responses of various PAH with the flame ionization and electron capture detectors (according to Cantuti *et al.* [68]).

Compound	Ratio ECD/FID
Anthracene	20.2
Fluoranthene	32.5
Pyrene	124.3
Benzo[a]fluorene	5.5
3-Methylpyrene	69.2
Benzo[ghi]fluoranthene	250.0
Benz[a]anthracene	267.2
Chrysene	1.5
Benzo[a]pyrene	343.5
Benzo[e]pyrene	310.0
Benzo[k]fluoranthene	180.2
Perylene	1.5

Admixture of oxygen or dinitrogen oxide (N_2O)(0.1-0.2 %) to the carrier gas enhances the ECD-response for various PAH by a factor up to 400 [69-71]. ECD is particularly useful for the determination of nitro-, halo- and azaarenes.

For the detection of nitrogen-containing compounds such as nitro- and azaarenes as well as aromatic amines nitrogen-specific (thermoionic) detectors with selectivity factors of about 10^4 are available which may also be used in parallel with FID. Furthermore, there are flame photometric detectors (FPD) commercially available which allow a selective detection of sulfur-containing polycyclic aromatic compounds (thiaarenes). Other detectors such as the micro-coulometric sulfur detector (MCD), the photoionization detector (PID) and the Hall electrolytic conductivity detector (HECD) with a high sensitivity and selectivity against carbon have also successfully been applied for the determination of thiaarenes.

In principle, spectroscopic detection as used routinely in HPLC may also be adapted to gas chromatography. A GC-UV combination has been described by Novotny *et al.* [72] and several approaches have been undertaken for a combination of GC with fluorescence detectors. Technical and economical circumstances prevented a more general application of these devices. This also holds for detector systems based on the phosphorescence, luminescence and low-temperature luminescence of PAHs although very sensitive and highly substance-specific signals may be obtained.

Recently, cryotrapping gas-chromatography-Fourier transform infrared spectrometry has become a useful method for the determination of PAHs which can distinguish between the parent PAH and their substituted derivatives [73]. Detection limits of about 1 ng and less have been reported for benzo[e]pyrene and chrysene [74].

A microwave induced atomic emission spectrometry detector in combination with GC (GC-AED) was described already in 1965 [75]. With regard to the limited precision [76,77] and sensitivity which is similar to that of other commonly used methods (nanogram range) it may be doubtful at present whether this device will find a wider application for the determination for PAHs.

Mass spectrometry is an ideal instrumental complement to high-resolution capillary gas chromatography and provides information on the molecular structure supplementary to UV and fluorescence spectrometry. Electron impact fragmentation is widely used for GC/MS coupling with both magnetic and quadrupole instruments. Full mass spectra may be obtained when high energy ionization (60-80 eV) is applied whereas activation energies of 8-20 eV for most PAHs result in molecular ions exclusively. Using modern equipment, full spectra can be recorded with 50-100 pg phenanthrene and about 100-500 pg benzo[a]pyrene. Sensitivity can be markedly enhanced (by factor of 100 and more) using the multiple or single ion mode.

For the identity and purity check of reference materials, the direct inlet mode is strongly recommended since high boiling impurities may escape detection by GC/MS combination.

Thiaarenes and oxaarenes may be readily recognized from their specific mass spectral fragmentation as indicated in Table 12. Similar fragmentation patterns have been observed for azaarenes [79].

Supplementary to electron-impact, chemical (negative and positive) ionization mass spectrometry (CIMS) is applied in PAH analysis with argon, argon-methane, oxygen and ammonia as reagents. Chemical ionization with NH_3/ND_3 has been successfully used for the characterization of aromatic amines and azaarenes [80]. Very high sensitivities for nitro-PAH have been reported using electron-capture negative ion mass spectrometry [81]. Tandem mass spectrometry [82] also appears to be an efficient and sensitive method (pg-range) for structural elucidation of polycyclic aromatic compounds as has been demonstrated by Wood *et al.* [83] for some sulfur-containing fuel constituents.

A rapidly growing interest in supercritical fluid chromatography (SFC) linking the HPLC- and GC-techniques (for review see ref. [84]) for the separation of PAHs is evident. Although still in a developmental phase, this technique seems to promise interesting results especially for the separation of high-boiling PAHs as shown for soot extracts by Kuei *et al.* [85]. Many peaks eluting after coronene could be detected when a 10 m x 50 μm quartz capillary coated with 50 % n-nonylpolysiloxane was eluted with hypercritical ammonia.

Apart from parent PAHs, thiaarenes, oxaarenes, azaarenes and their alkyl derivatives, other related structures have found increasing interest recently. The high mutagenic potential of various nitro-PAH resulted in numerous analytical methods for their determination [16]. There is extensive literature on the analysis of PAH metabolites mainly by HPLC and GC methods [85] to which the interested reader is referred.

Table 12 Key-ions for the characterization of unsubstituted and monomethylsubstituted PAH, thiaarenes and oxaarenes (from ref. [78]).

	PAH	Me-PAH	S-PAC	Me-S-PAC	O-PAC	Me-O-PAC
M	+	+	+	+	+	+
M-1	<M-2	>M-2	<M-2	>M-2	<M-2	>M-2
M-2	>M-1	<M-1	>M-1	<M-1	>M-1	<M-1
M-16	-	-	-	-	(+)	-
M-17	-	-	-	-	-	+
M-26	+	-	-	-	-	-
M-27	-	+	-	+	-	-
M-29	-	(+)	-	-	+	-
M-30	-	-	-	-	-	+
M-32	-	-	+	-	-	-
M-33	-	-	-	+	-	-
M-45	-	-	+	-	-	-
M-46	-	-	-	+	-	-
M-55	-	-	-	-	+	-
M ⁺ /2e	+	+	+	+	+	+
(M-1)/2e	-	+	-	+	-	-
(M-16)/2e	-	-	-	-	+	-
(M-17)/2e	-	-	-	-	-	+
(M-26)/2e	+	(+)	+	-	-	-
(M-27)/2e	-	+	-	+	-	-
(M-29)/2e	-	-	-	-	+	-
(M-30)/2e	-	-	-	-	-	+
(M-33)/2e	-	-	-	+	-	-
(M-45)/2e	-	-	+	-	-	-
(M-46)/2e	-	-	-	+	-	-
(M-55)/2e	-	-	-	-	+	-

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24.

Method validation for the determination of dioxins

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Polychlorinated dibenzo(p)dioxins (PCDD) and dibenzofurans (PCDF), both often referred to as "dioxins", are two groups of tricyclic aromatic compounds with very similar chemical structures (Fig.1) and therefore with very comparable chemical properties.

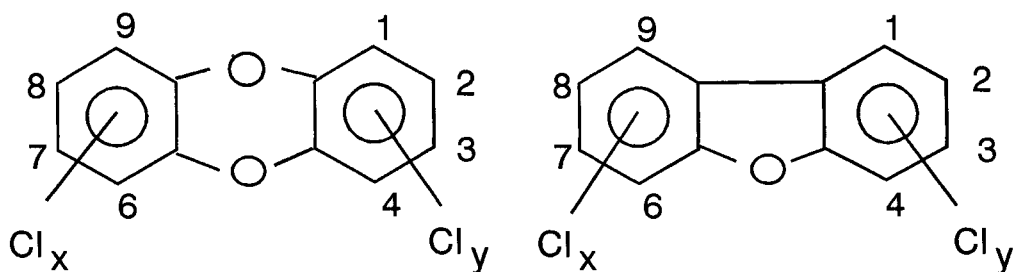


Figure 1: Chemical structure of PCDD and PCDF

All compounds involved are solids with high melting and boiling points, which show a very limited solubility in water, a good solubility in fat and medium polarity solvents and an extreme stability in time in a broad range of situations.

Depending upon the degree of chlorination and the positions of the chlorine atoms, 210 different compounds are possible (Table 1).

Table 1: Homologue groups and numbers of isomers for PCDDs and PCDFs

Chlorine content	Number of PCDD isomers	Number of PCDF isomers
Mono-	2	4
Di-	10	16
Tri-	14	28
Tetra-	22	38
Penta-	14	28
Hexa-	10	16
Hepta-	2	4
Octa-	1	1
TOTAL	75	135

None of these compounds has ever been manufactured intentionally for commercial use. They always were, and still are, generated as inevitable by-products in a large variety of chemical reactions and a broad range of combustion processes.

Proven examples are the chemical synthesis of polychlorinated aliphatics [1], phenols [1,2], biphenyls [2,3] and pesticides [4], as well as the incineration of municipal waste [1,2,5-11], hazardous waste [11], hospital waste [1,11] and sewage sludge [12], wire reclamation incineration [12], metal recycling [1], the combustion of coal [13-15], wood [16] and waste oil [14], and the combustion of leaded fuel in motor vehicles [17-19].

A few examples of suspected but not yet fully proven sources are the combustion of landfill gas [1], biogas [1] and peat [11], the production of chlorine [20,21], and a broad series of chlorinated organic and inorganic chemicals [22], sintering processes [1,11], asphalt mixing [1], carbon reactivation [23], catalyst regeneration [24] and certain soil cleaning processes [1].

As a consequence, these compounds rather easily find their way into the environment, into the food chain and finally into man. Polychlorinated dioxins and dibenzofurans have indeed been identified in air [25,26], soil [27], water [22,28], sediments [29-32], plants [33,34], fish, meat, cow's milk [35], human adipose tissue and breast milk [35,36], and other biological media. It is actually estimated that more than 90 % of the daily intake for an average person results from food and that less than 5% comes through inhalation.

The concern for the human health effects of dioxins was initiated by the occurrence of a number of critical situations, in which there was an extreme pollution resulting from regrettable accidents (Seveso, Love Canal, Times Beach) or from the excessive use of contaminated herbicide formulations (Agent Orange). This concern, which was subsequently propagated by the fact that dioxins were found to constitute an almost ubiquitous low level environmental contamination problem, resulted in numerous efforts in the field of toxicological evaluation and the assessment of the associated human health risks [37,38].

In spite of the many efforts, there is actually still no general consensus whatsoever amongst researchers as to the chronic toxic effects of dioxins on man, and the exact mechanisms of action are still far from being understood. The only more or less definite results consist in the general agreement that:

- only those 17 compounds with chlorine substitution at the 2, 3, 7 and 8 positions exhibit toxic effects;
- within this group, 2,3,7,8-tetrachlorodibenzo-p-dioxin exhibits the highest toxicity. It is even the most potent carcinogen ever tested on animals. It is 50 times more potent than aflatoxin B1 and 50,000,000 times more potent than vinylchloride;
- the toxicity of the other members of the group relative to that of 2,3,7,8-T4CDD varies over a very broad range as indicated by the so called International Toxicological Equivalence Factors (TEF, Table 2) [39].

Table 2: International Toxicological Equivalence Factors for the so called "dirty seventeen".

PCDD	I-TEF	PCDF	I-TEF
2,3,7,8-T4CDD	1	2,3,7,8-T4CDF	0.1
1,2,3,7,8-P5CDD	0.5	2,3,4,7,8-P5CDF	0.5
		1,2,3,7,8-P5CDF	0.05
1,2,3,4,7,8-H6CDD	0.1	1,2,3,4,7,8-H6CDF	0.1
1,2,3,6,7,8-H6CDD	0.1	1,2,3,7,8,9-H6CDF	0.1
1,2,3,7,8,9-H6CDD	0.1	1,2,3,6,7,8-H6CDF	0.1
		2,3,4,6,7,8-H6CDF	0.1
1,2,3,4,6,7,8-H7CDD	0.01	1,2,3,4,6,7,8-H7CDF	0.01
		1,2,3,4,7,8,9-H7CDF	0.01
OCDD	0.001	OCDF	0.001

- all 2,3,7,8-chlorosubstituted compounds are extremely stable and show accumulation effects so that even the smallest level of pollution could constitute a potential risk.

In spite of the remaining scientific uncertainties as to the toxicity of dioxins to man, several countries and organizations concluded that, until proven otherwise, the dioxin problem should not be underestimated and decided to work out efficient dioxin control schemes and to develop appropriate regulations aiming at the reduction of dioxin emissions and at the limitation of the daily intake of dioxins by their citizens [40].

It is obvious that such decisions could have a number of extremely important worldwide social and economic consequences. Therefore they must be based on, and supported by, up-to-date and proven analytical methodologies. In order to fulfil the very strict quality requirements necessary in this context, these methods should be accompanied by adequate procedures for quality assurance and quality control. It is evident that good laboratory practices must also be applied for sampling. Indeed, although it is beyond the scope of this review to include a detailed discussion about sampling methods, it must be highlighted at this point that representative sampling is an essential condition in order to get meaningful results. An elaborate discussion of sampling requirements and guidelines for use in environmental studies has been published by the American Chemical Society [41]. The European Committee for Standardization (CEN) is preparing a standard regarding the sampling of flue gas emissions for PCDD- and PCDF-determinations.

24.1 Review of existing methods for the determination of PCDD and PCDF

The analytical history of dioxins and dibenzofurans does not differ that much from that of other environmental toxins. Once the problem was generally recognized, detection and unambiguous identification in a broad range of different matrices became the first problem. As a consequence, early analytical methods were directed almost exclusively towards the qualitative or semiquantitative screening of samples for the presence of the different classes of PCDD and/or PCDF (group specific analyses). Soon afterwards, the almost ubiquitous occurrence of both groups of pollutants as well as their extremely potent toxicity and the relatively large variation in toxicity between different isomeric groups or even between different members of the same isomeric group, stressed the need for more accurate quantitative and isomer specific measurements. Nowadays, the value of the early pioneering methods has become rather limited. As a consequence the review presented here will be restricted to the state of the art regarding the "modern" analytical methodologies for the quantitative determination of dioxins and dibenzofurans.

In general, the analytical methods used for the determination of dioxins and dibenzofurans consist of three consecutive stages:

(i) *Extraction of dioxins and dibenzofurans from the sample matrix*

In order to be efficient, the extraction step must often be preceded by some kind of chemical treatment of the sample matrix such as destruction or digestion;

(ii) *Clean-up of the crude extract*

The clean-up aims at the elimination of all co-extractants which might hamper or even completely obstruct the final quantitative determination of PCDD and PCDF in the extract. The degree of clean-up required obviously depends upon the selectivity offered by the final instrumental technique used for analysis.

(iii) *Instrumental analysis*

A number of strict requirements must be taken into account regarding the instrumental technique selected for final analysis. Ultra-sensitive detection, elimination of interferences and accurate quantitation are key factors in order to be successful.

The literature describes a large number of analytical procedures for the quantitative determination of dioxins and dibenzofurans. However, most of these procedures are very similar and only differ from one another in a number of details such as the digestion and extraction reagents used or the types of reagents and adsorbents applied for clean-up and even the sequence of their application. As a consequence, it seems more appropriate to describe the existing analytical methodology for dioxin and dibenzofuran analysis in a modular way.

24.1.1 Extraction

24.1.1.1 Liquids

When dealing with liquids such as surface waters, ground waters, effluents, condensates and liquid chemical wastes, liquid/liquid extraction is the most suitable extraction technique. In principle, any technique allowing a good homogenization of both the water and the solvent layers can be applied.

Relatively clean water samples can be extracted directly using dichloromethane [42-46], n-hexane [47,48], n-pentane [43-45,49,50], petroleum ether [51,52] or an appropriate mixture of polar and/or non polar solvents (e.g. 15 % dichloromethane in hexane [43] or 5 % benzene in dichloromethane [53]). Adjustment of the pH of the water sample is not required as no measurable effect on extraction efficiency, which easily reaches 90 to 100 %, could be detected [42].

Complex samples, such as heavily loaded waste waters and especially chemical wastes, are extracted with n-hexane while partitioning against a mixture of water and acetonitrile [54] or an alkaline solution [55,56]. In this case, the extraction efficiencies reported in the literature vary between 50 % and 100 % [44,45].

The main disadvantage of liquid/liquid extraction however is that often multiple extractions are needed, entailing the use of relatively large amounts of solvents, in order to reach the desired extraction efficiencies. The subsequent elimination of these solvents is time consuming and may result in problems of compound loss or the introduction of external contamination. This is especially true in the ultra-trace analysis of dioxins. These problems can be minimized by using one of the continuously operating liquid/liquid extraction devices available on the market. In these systems, the volume of solvent needed is greatly reduced, whilst the number of extraction steps per unit of time is increased appreciably. Their use should be recommended.

24.1.1.2 Soils and sediments

Soils and sediments are generally dried first. This can be done by spreading the sample on a glass plate and just leaving it overnight at room temperature, by grinding under liquid nitrogen or by freeze drying. Drying is sometimes replaced by mixing with appropriate amounts of anhydrous sodium sulphate [53,57,58]. Elimination of the water from the particles of soil or sediment is extremely important when the final extraction solvent is not miscible with water. When readily water miscible solvents are used, the drying step becomes much less critical as the contact of the solid with the solvent is not hampered by the aqueous phase.

Subsequently, the dried samples are generally Soxhlet extracted using benzene [44,59], toluene [60-64], a mixture of 5 % benzene in dichloromethane [53,65], or a 50 % mixture of acetone in n-hexane [57,64]. Extraction times range from 16 h to 48 h and the extraction efficiencies reported are between 50 % and 100 %.

Depending upon the type of soil or sediment and its history, Soxhlet extraction is sometimes preceded by an acid treatment using sulfuric [66], or hydrochloric acid [50,58] in order to increase the extraction efficiency. Acid treatment is thought to open the structure of the solid material and therefore to make the surfaces more accessible for the extracting solvent [58,67] (Table 3).

Table 3: Spike recoveries from a bay sediment with and without HCl pretreatment.

Isomer	Without HCl treatment			With HCl treatment		
	\bar{R}_n	N	CV%	\bar{R}_s	N	CV%
2,3,7,8-TCDD	45	16	15	70	12	10
2,3,7,8-TCDF	25	16	10	60	12	10
OCDD	30	14	15	90	20	10

\bar{R}_n , \bar{R}_s : average recoveries in neutral and acid conditions.

N: the number of replicate extractions on which the averages are based.

CV %: coefficient of variation in %.

24.1.1.3 Solid sorbents and paper

Solid sorbents such as XAD-2 [46,50,68], Florisil [57] and polyurethane foam (PUF) [62,68-71] are customized adsorbents for sampling dioxins and dibenzofurans from air and stack emission gases. Different types of bleached paper are suspected to be dioxin carriers because of the chlorine used in the bleaching process [72,73]. The samples are extracted directly in a Soxhlet apparatus using most frequently dichloromethane for Florisil, toluene for XAD-2 or PUF and ethanol for paper. Although it is tempting to perform soxhlet extractions overnight, and therefore to limit the extraction time to approximately 16 h, one must realize that this can cause slightly reduced extraction efficiencies (Table 4), a problem which might be worth taking into consideration when performing ultra-trace analysis. Extraction times of at least 20 h generally yield extraction efficiencies of over 90 %.

Table 4 Average Soxhlet extraction efficiencies for the PUF - toluene combination, after 16 and 20 h.

Compound	16 h extraction time			20 h extraction time		
	\bar{R}_{16h}	SD	N	\bar{R}_{20h}	SD	N
T4CDD	59	3.2	32	99	3.6	25
H6CDD	77	4.1	32	90	3.3	25
OCDD	74	7.6	32	91	3.4	25

\bar{R}_{16h} and \bar{R}_{20h} : average recoveries after 16 and 20 h.

SD: standard deviation of the mean.

N: number of samples extracted.

24.1.1.4 Soot, particulates and fly ash

The analytical methodology used for the extraction of dioxins and dibenzofurans from soot, airborne particulates and fly ash consists of a preliminary acid treatment, using 1 mol.l⁻¹ hydrochloric acid, followed by Soxhlet extraction in toluene for at least 24 h [3,74-77]. The preliminary acid wash, which as stated before is thought to open the particle structure, is extremely important for these samples, probably because of the extreme thermal conditions in which the samples are generated. The choice of toluene as the extraction solvent instead of benzene is merely based on toxicological considerations. Other solvents such as dichloromethane, chloroform or a mixture of n-hexane and acetone are much less effective. Extraction efficiencies for PCDD and PCDF using different procedures, as evaluated by Lustenhouwer *et al.* [78], are summarized in Table 5. Lustenhouwer proved that, when acid treatment is included, an overall extraction time of 24 h results in extraction efficiencies of over 97 % (Table 6) for all dioxins and dibenzofurans [79].

Table 5 Extraction efficiencies in % for PCDD and PCDF in fly ash, using different procedures and different solvents [78]

Method	% PCDD	% PCDF
Acid (HCl) + Soxhlet/toluene (35 h)	100	100
Soxhlet/dichloromethane (35 h)	6	11
Soxhlet/chloroform (35 h)	13	20
Shaking in dichloromethane (4 extracts)	1	2
Ultrasonic acetone/n-hexane (4 extracts, each 5 min)	1	1
Fly ash suspended in 48 % HF + toluene liquid/liquid	9	16

Table 6 Extraction efficiency in % as a function of total extraction time for a fly ash sample extracted with toluene after acid treatment. The values are given in % of the amount extractable after 72 h [79]

Congeners	After 24 h	After 48 h
T4CDD	97.0	99.3
P5CDD	97.4	99.4
H6CDD	98.4	99.7
H7CDD	99.3	99.9
OCDD	99.5	100
T4CDF	97.7	99.4
P5CDF	98.4	99.7
H6CDF	98.6	99.7
H7CDF	99.4	99.9
OCDF	99.5	99.7

Table 7: extraction methods used for different biological samples.

Extraction procedure			
Pretreatment	Extraction	Application	Refs.
Homogenization	extraction by agitation in chloroform/methanol or in acetone/n-hexane	fish human fat	[80,81] [82]
None	in batch extraction with dichloromethane with homogenization	tissue	[83,84]
None	in batch extraction with acetonitrile with homogenization	human tissue	[85] [48]
None	in batch extraction with benzene/n-hexane 1/1 with homogenization	animal tissues fish	[65]
None	multiple extraction in dichloromethane/methanol with homogenization	fish	[86]
Addition of acetone, NH ₄ Cl, K-oxalate or Na-oxalate	liquid/liquid extraction with n-hexane, ethanol, diethyl-ether, pentane or petroleum ether or a combination of them	meat products cow's milk human milk	[87,88] [89,90]
Addition of n-hexane	in batch extraction with acetonitrile	cow's milk	[91]

Table 7: continued.

Extraction procedure			
Pretreatment	Extraction	Application	Refs.
Addition of anhydrous sodium sulphate, silica gel, sea sand or a combination of these and grinding	extraction by shaking with n-hexane/acetone 2/1, dichloromethane or benzene n-hexane	blood plasma, meat products, human organs	[86,88] [59] [92]
Addition of anhydrous sodiumsulphate and sea sand + grinding	in column extraction with dichloromethane, dichloromethane/cyclohexane 1/1 or n-hexane/aceton 2/1.	fish, liver seal tissue	[90,92,93] [94,95,96]
Freeze drying	Soxhlet extraction for 48 h in toluene	fish tissue	[97]
Saponification (KOH or NaOH-treatment) at room temperature for 2 to 3 h or under reflux conditions.	extract with n-hexane, benzene.	liver, adipose tissue, milk fish	[48,98,99] [100] [101]
Mixing of blood with water	Load on Chem-Elut column (modified silica), elution with n-hexane/isopropanol 3/2	whole blood	[102]

24.1.1.5 Biological samples

There is a wide diversity of extraction methods applied to biological samples such as fish, liver, adipose tissue, blood plasma, whole blood, cow's milk, breast milk and fat. In general, these methods can be grouped into three categories: those aiming at the direct extraction of the dioxins from the fatty matrix, those extracting the dioxins after destruction and/or elimination of the lipids present and finally those aiming at a complete extraction of the fat which has then to be destroyed and removed afterwards. Table 7 summarizes a number of these methods relative to the matrices to which they were applied.

Data on the extraction efficiencies for these methods are extremely scarce. This is due to the fact that, because of the complexity of the resulting extracts, a straightforward determination of the spikes in the uncleaned extract is virtually impossible when using methods currently available in dioxin laboratories. This problem can be solved by using spikes labeled with radioactive isotopes such as ^3H or ^{14}C , which can be easily determined in both the extract and the remaining sample by using the appropriate radiation counting devices. Even then, there will always be the question of the validity of spiking for the determination of extraction efficiencies, especially in the case of biological samples, because it is not at all evident that both the spikes and the native dioxins are present in the same physical state. As a consequence most authors do not make the effort and just list the overall method recoveries which, depending upon the sample and the nature of the clean-up performed, can be more or less indicative for the efficiency of the extraction procedure used. The recoveries reported are generally between 50 % and 100 % [59,82,85,86,89,94,100-104].

24.1.2 Clean-up

24.1.2.1 Acid / base clean-up

Treatment of the extract with concentrated sulphuric acid results in the destruction and elimination of basic and oxidizable co-extractants, while the action of bases leads to the removal of the acidic components present [45,47,57,62,65,70,72,75,80,81,84,87, 94,101]. Both treatments require that the extracting solvent is first exchanged for n-hexane.

24.1.2.1.1 In batch treatment.

This clean-up step is applied to moderately (e.g. soils and sediments) and heavily loaded extracts (e.g. biological samples). The bulk of the basic and oxidizable compounds is removed by treating the extract a number of times with concentrated sulphuric acid. The sulphuric acid washing is repeated until the acid phase remains colourless. Samples possibly containing relatively large amounts of acid co-extractants are often treated using aqueous solutions of KOH or NaOH. Although these batch treatments have a higher capacity, they have a limited efficiency.

24.1.2.1.2 Liquid chromatography on acid/base modified silicagel.

Traces of basic, oxidizable and acidic compounds, present in original extracts or remaining after the batch treatment, are eliminated, after concentration of the remaining hexane solution to an acceptable volume, by transferring the concentrate to a liquid chromatography column loaded with acid and/or base modified silica gel,

followed by elution with n-hexane (Fig. 2). The acid modified silica gel is prepared by mixing 44 g of concentrated sulphuric acid with 56 g of previously cleaned and activated silica gel in an appropriately sized and tightly closed glass bottle. The mixture is shaken gently until all clumping has disappeared. The glass bottle can be stored in a desiccator. The base modified silica gel is prepared in the same way by mixing 33 g of a NaOH solution with 67 g of pre-cleaned and activated silica gel. Both packings can be used individually or in combination, loading them in the same column as consecutive layers separated by normal silica gel. Regular column diameters used for this clean-up range from 1 to 2 cm. The required column length or in other words the required capacity is difficult to predict as it depends upon the amount of coextractants to be removed. When using sulphuric acid treated silica gel, which turns black upon reaction, the LC-step should be repeated if a completely black column remains after elution. Whilst the column capacity remains sufficient, at least the lower part of the column will keep its white colour. For the silica gel loaded with NaOH there are no visual indications.

24.1.2.2 *Treatment with AgNO₃*

Silver nitrate is used in order to eliminate DDE, chlorinated aliphatic hydrocarbons, sulfides and sulfur [105]. This clean-up is most frequently performed by liquid chromatography using silica gel loaded with AgNO₃ as the adsorbent and n-hexane as the eluting solvent [45,50,68,70,75,101]. The AgNO₃-modified silica is prepared by mixing, in an appropriately sized and hermetically closed glass bottle, 67 g of previously cleaned and activated silica with 33 g of an aqueous solution containing 300 g.kg⁻¹ of AgNO₃. The mixture is then shaken gently until a free flowing powder results. Due to the extreme sensitivity of the AgNO₃ to oxidation, the AgNO₃ modified silica should be prepared freshly for each experiment. Additionally, the recipient used for the preparation as well as the column used for the actual clean-up should be shielded from the light. No fixed advice can be given as to the dimensions required for the LC-columns used for this purpose. Columns are often between 5 and 10 mm in diameter and between 100 and 400 mm in length. If properly shielded from the light, a column which is not overloaded will show a more or less dark grey colour in the reacting zone but will stay nearly white at the bottom.

24.1.2.3 *Liquid chromatography on silica gel*

Liquid chromatography on silica gel is applied in order to eliminate polar compounds such as acids, phenols, chlorinated phenols and polychlorophenoxyphenols [3,47,54,57,66]. Before use, the silica must be cleaned and activated. Cleaning can be performed by washing the silica in different solvents. Methanol followed by methylene chloride is frequently used for this purpose. Activation is performed by subsequent heating of the washed and air dried silica in a tube furnace under a flow of dry nitrogen. Activation time and temperature are not very critical parameters in the case of dioxin analysis. Heating at 180 °C for about 2 h, or at 160 °C for about 3 h, should be sufficient in order to obtain a properly activated silica. The only condition, which should be checked regularly, especially when working with a stock of activated silica stored over longer periods of time, is that the final water content of the silica must be ≤ 3 % by mass. The sample, dissolved in n-hexane, is loaded onto the column which is then eluted with an appropriate volume of methylene chloride/n-hexane 20/80. Column diameters are normally between 5 and 10 mm. The required

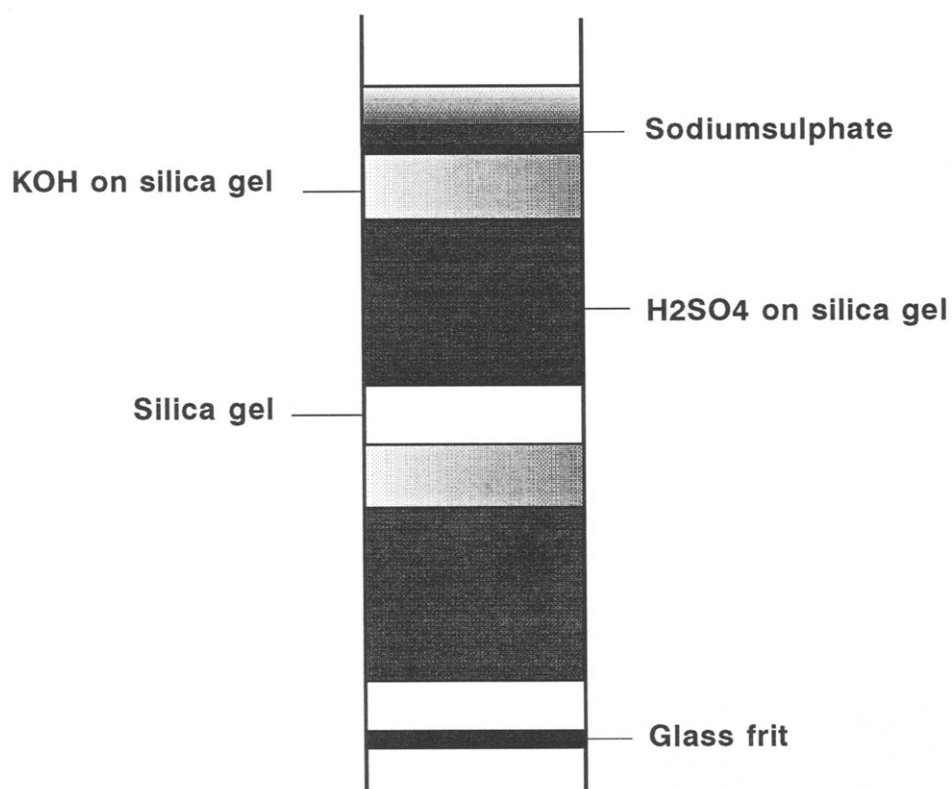


Figure 2: Mixed column containing consecutive layers of base modified, acid modified and neutral silica gel.

column capacity and the solvent volume necessary for a complete elution of the dioxins depend upon the sample size and the amount of polar co-extractants present.

If necessary, the polar compounds, which stay on top of the column, can be recovered by elution with acetone.

24.1.2.4 *Liquid chromatography on Florisil*

Liquid chromatography on Florisil is employed in order to effect a separation between the polychlorinated biphenyls and the dioxins present in the samples [53,63,80-82,88]. Cleaning and activation of Florisil is performed using the same reagents and heating/purging equipment as described earlier for silica gel. Activation is generally enforced by heating up to 225 °C for 24 h. In most cases where Florisil clean-up has been used, relatively large columns have been used (e.g. 11 mm diameter and 500 mm length) in combination with relatively large volumes of solvents for elution (generally different mixtures of methylene chloride and n-hexane). Bearing in mind the difficulties that might be encountered in trace and ultra-trace analysis, when one has to evaporate large quantities of solvent, it seems reasonable that the use of Florisil in procedures for dioxin determinations is rather limited, especially because neutral, basic or acidic alumina, which will be described later, offer comparable possibilities in view of the elimination of polychlorinated biphenyls.

24.1.2.5 *Liquid chromatography on neutral-, basic- or acidic-alumina*

As one would predict from the huge amount of references, alumina is certainly the adsorbent used most frequently in clean-up procedures for dioxin analysis [45-47,50,57,59,60,62,65,66,68-71,75,79,83,84,88,89,91,94,95,97,98,101,103,106]. Liquid chromatography on alumina allows the elimination from the dioxin sample of chlorinated benzenes and naphthalenes, polychlorinated biphenyls, polychlorinated terphenyls and polychlorodiphenylethers. Certain members of these co-extractants, if present, require a combined clean-up on basic-, followed by acidic-alumina for their complete elimination [106]. Cleaning and activation of neutral-, basic- and acidic-alumina is performed using the same reagents and heating/purging equipment as described earlier for silica gel.

In the literature, multiple activation procedures have been reported, e.g. 16 h at 600°C, 4 h at 400 °C, 2 h at 300 °C, 16 h at 200 °C, 16 h at 130 °C, 16 h at 110 °C and "use as delivered" for basic alumina; 16 h at 200 °C and 16 h at 130 °C for acidic alumina; 18 h and 24 h at 225 °C for neutral alumina. In spite of these differences in activation, each of these procedures yields an alumina adsorbent able to do the clean-up for the types of samples studied. The only condition is that, depending upon the sample to be analysed, the alumina must be used in appropriate quantities and that specific solvent mixtures and solvent volumes are required for the elution of both the undesirable coextractants and the dioxins. As an example, a column of 5 mm internal diameter, containing 1 g of basic alumina activated for 16 h at 600 °C, has been used for the clean-up of incinerator effluents [47]. The column was first eluted with 7 ml of methylene chloride/n-hexane 3/97 in order to remove the undesirable co-extractants, followed by 20 ml of a 50/50 mixture of the same solvents for the recuperation of the dioxins. On the other hand, a column of 10 mm internal diameter, made up with 10 g of the same support was successfully used for dioxin analysis in paper pulp samples [106]. The undesirable impurities were eluted first using 30 ml of n-hexane and 50 ml of methylene chloride/n-hexane 8/92, while the dioxins were collected in a third

fraction using 45 ml of pure methylene chloride. These examples are an illustration of the flexibility offered by alumina adsorbents for sample clean-up in dioxin analysis. However, it should be highlighted at this point that the word "flexible" is not synonymous with "unimportant as to its activity". Once the clean-up procedure has been established, the activity of the adsorbent should be controlled very closely using a reliable activity check.

24.1.2.6 *Gel permeation chromatography*

Recently, gel permeation has been introduced as a valid alternative for the removal of lipids and proteins from dioxin extracts. This clean-up, though especially important for the analysis of food and biological samples, can also facilitate the clean-up of soils, sediments and waste waters which might contain important amounts of biological materials. In gel permeation chromatography, the relatively large fat and protein molecules elute much faster than the dioxin fraction. Columns loaded with Bio-Beads S-X3, first conditioned in and then eluted with a 1/1 mixture of cyclohexane/ethylacetate are used most frequently for this purpose [88]. Depending upon the load in organic materials of the samples analysed, these columns can be reused several times. Again there is no general rule as to column diameter and column capacity, which depend exclusively on the types and the amounts of the biological material present.

24.1.2.7 *Liquid chromatography on activated carbon*

Activated carbon selectively adsorbs polychlorinated dioxins, polychlorinated dibenzofurans, comparable coplanar compounds and o,o-substituted polychlorinated biphenyls. All other compounds such as DDE, ortho-substituted polychlorinated biphenyls, polychlorinated methoxybiphenyls, polychlorinated diphenylethers and polychlorinated methoxydiphenylethers, can be readily eluted from a carbon column when using the appropriate solvents or solvent mixtures. Several column packings and methods have been used for carbon clean-up [57,59,64-66,71,83,84,87,89,94,95,107]. These comprise pure carbon (Carbopack C or Carbosphere), as well as mixtures of carbon with suitable carriers (5 % of carbon AX-21 mixed with silica gel, 15 % of Carbopack C on Celite 545, 8 % of Amoco PX-21 on Celite 545 or 50 % of carbon on glass fibres). The solvents used for the preliminary elimination of undesirable co-extractants generally are 5 to 10 % (V/V) mixtures of benzene or toluene in n-hexane for carrier diluted columns, and 1/1 (V/V) mixtures of methylene chloride/n-hexane or even pure toluene for pure carbon columns. The dioxin recovery from mixed columns is accomplished by increasing the toluene concentration in the n-hexane to 50 % or even by using pure toluene. The dioxin recovery from pure carbon columns requires reverse elution, sometimes even under reflux conditions, with pure toluene.

It will be evident that dioxin-like co-planar compounds and o,o-substituted polychlorinated biphenyls are not eliminated by a carbon clean-up. If such compounds are suspected to be present, the inclusion of a single or even multiple alumina clean-up (basic alumina followed by acidic alumina) will be necessary [107].

24.1.2.8 *High pressure liquid chromatography*

Normal and reversed phase high pressure liquid chromatography (HPLC) were originally introduced as a way to separate the total dioxin fraction into different sub-fractions which then allowed interference free determination of specific dioxin congeners such as 2,3,7,8-tetrachlorodibenzo-p-dioxin [51,108,109].

However, nowadays, the interest of researchers is directed towards the determination of all 2,3,7,8-chlorinated dioxins and dibenzofurans, a development which has been made possible by direct gas chromatographic analysis of the dioxin mixtures on modern polar capillary columns. As a consequence, the applications of HPLC in the field of dioxin analysis have become limited and are actually restricted to special problems requiring the elimination of very special interferences [110,111,112,113].

24.1.2.9 Clean-up procedures

The combination of these clean-up modules into a fixed clean-up procedure obviously depends upon the type of sample at hand as well as upon the accompanying contaminants. The following schemes are an illustration of a number of combinations often used for specific purposes (Figs. 3-8).

24.1.3 Instrumental analysis

Some very strict requirements must be fulfilled by instrumental methods used for dioxin and dibenzofuran analysis. The complex nature of the samples to be analysed, as well as the large number of different isomers belonging to the dioxin family, demand extreme selectivity. Selectivity becomes even more important when taking into account that increased instrumental selectivity almost always has the positive effect of appreciably shortening the time consuming and risk prone clean-up procedures required for the analysis. Additionally, in view of the extremely low concentrations that have to be monitored, there is the need for ultra-sensitive detection. It is generally acknowledged that capillary gas chromatography in combination with mass spectrometric detection is actually the only technique that offers both the adequate sensitivity and selectivity for reliable dioxin determinations.

24.1.3.1 Capillary gas chromatography

The gas chromatographic separation of the seventeen 2,3,7,8-chlorinated dioxins and dibenzofurans of interest ("dirty seventeen") from other dioxin isomers or accompanying impurities can be accomplished using different types of capillary columns. An overview of the possibilities offered by different types of liquid phases was published recently by Ryan *et al.* [114]. In practice, the separation required and thus the column used for specific samples will depend upon the dioxin pattern and the accompanying impurities present in the cleaned-up sample. So, the quantitative determination of all 2,3,7,8 chlorinated dioxins and dibenzofurans present in cow's milk can be accomplished by using a single chemically bonded non polar column, while the same determination in a fly ash requires the use of at least two different polar capillary columns.

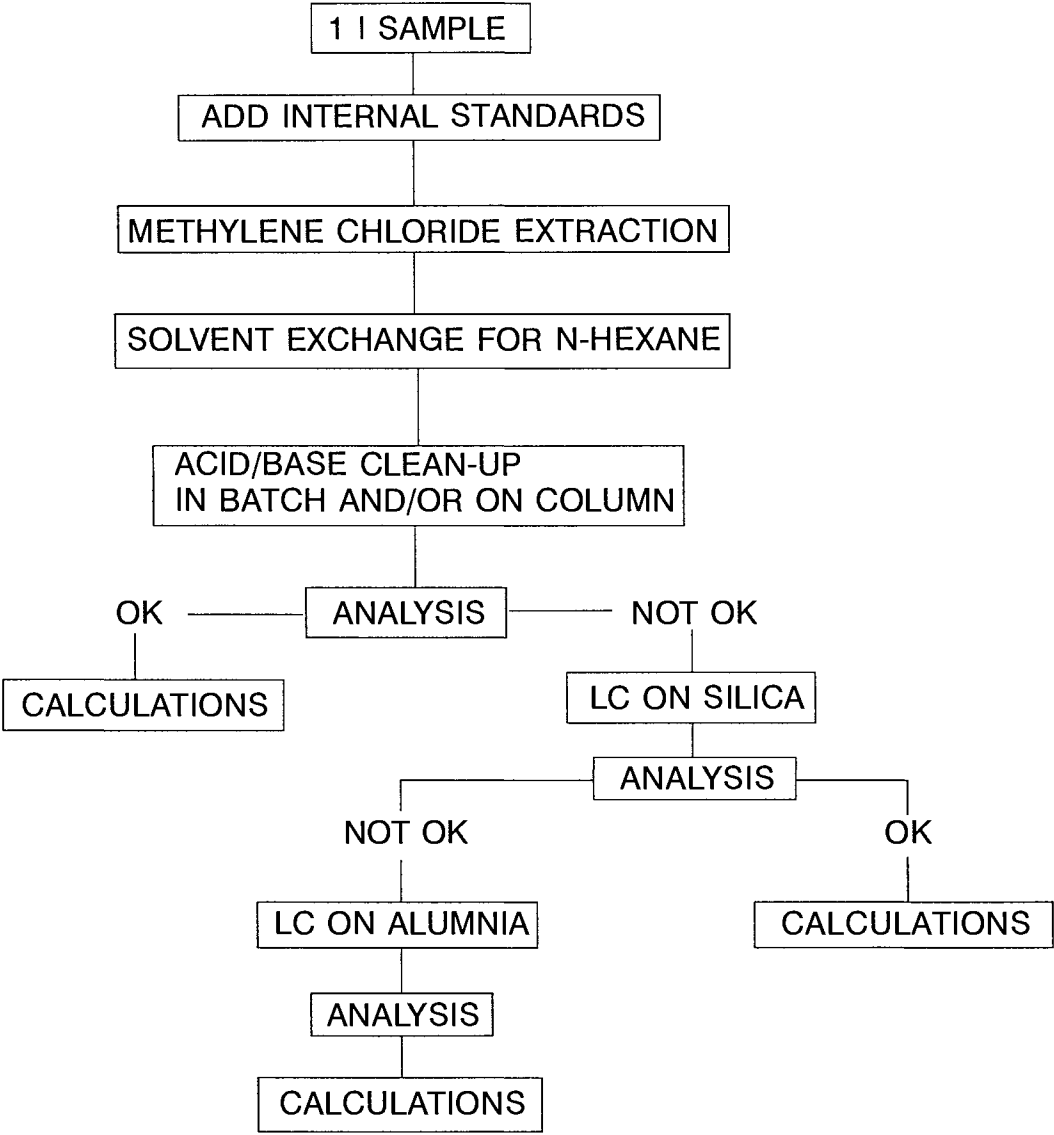


Figure 3: Clean-up scheme for industrial and municipal waste waters.

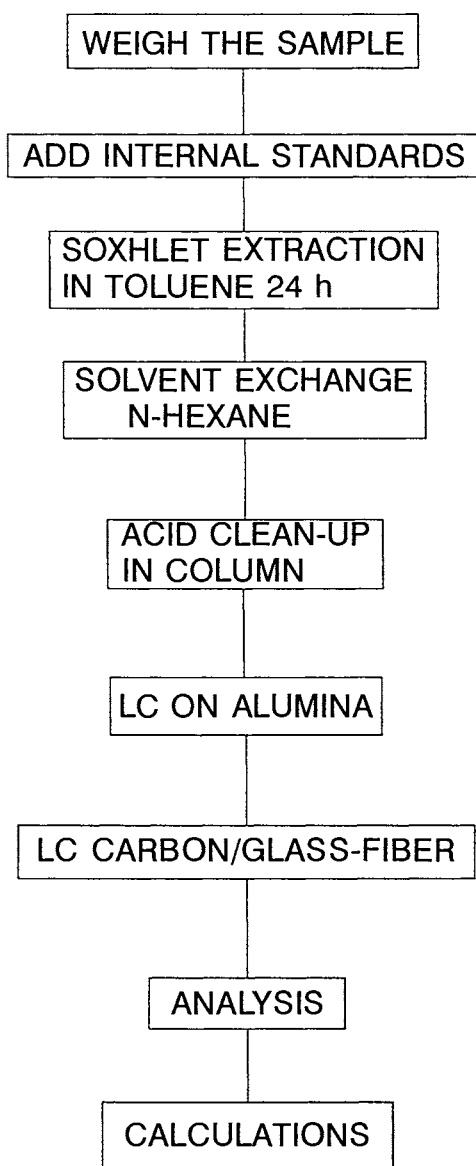


Figure 4: Clean-up scheme for soot.

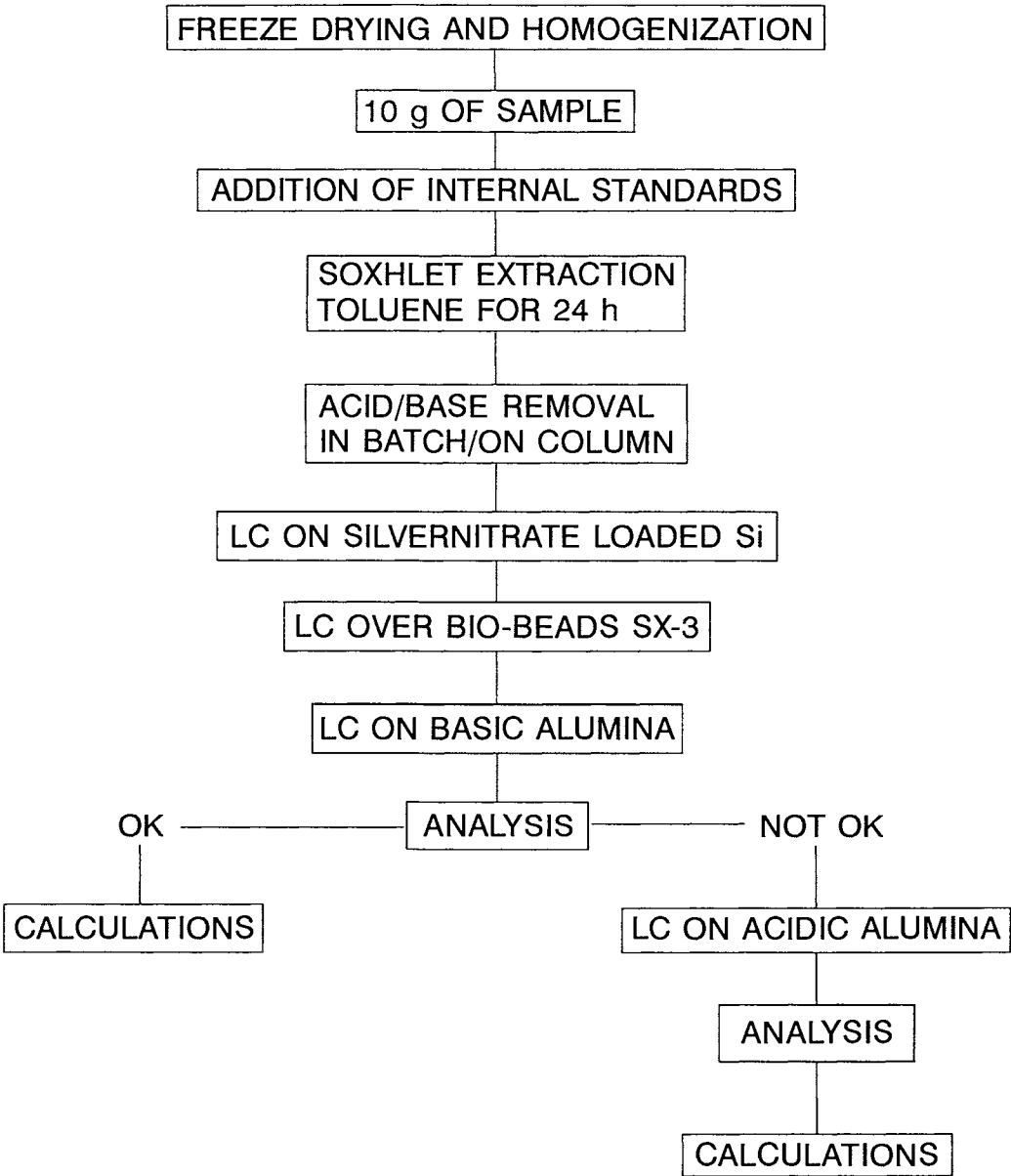


Figure 5: Clean-up scheme for soils and sediments.

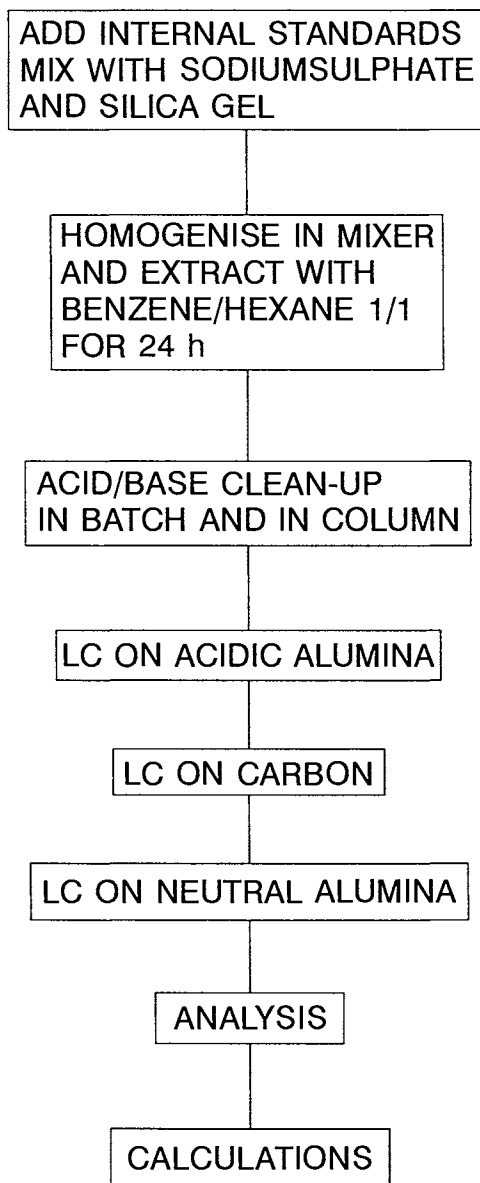


Figure 6: Clean-up scheme for biological samples.

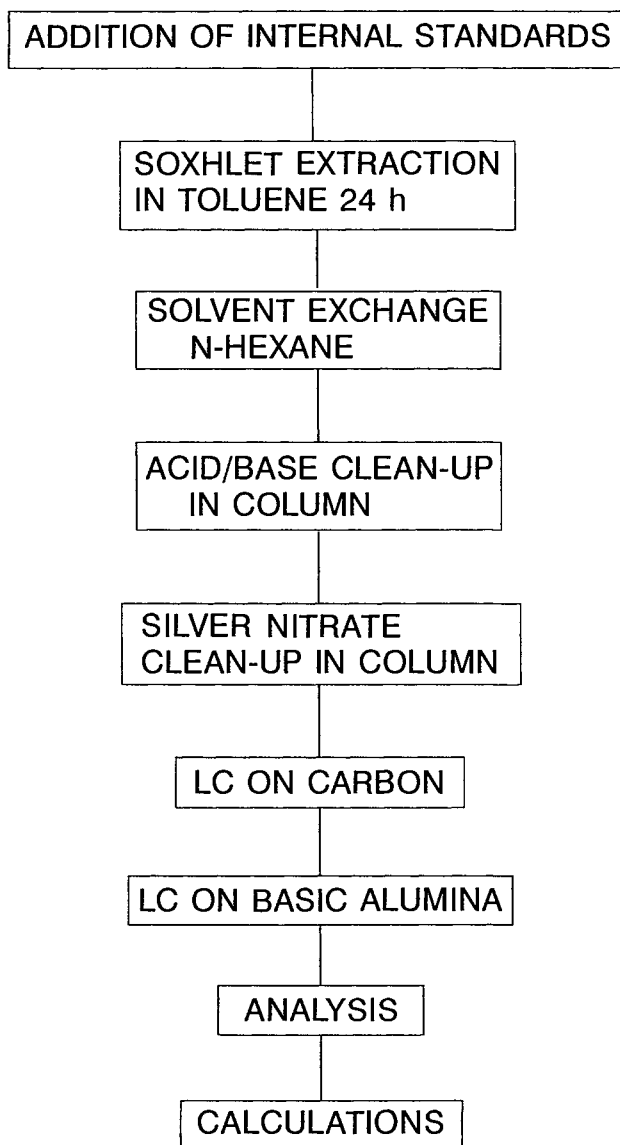


Figure 7: Clean-up scheme for polyurethane.

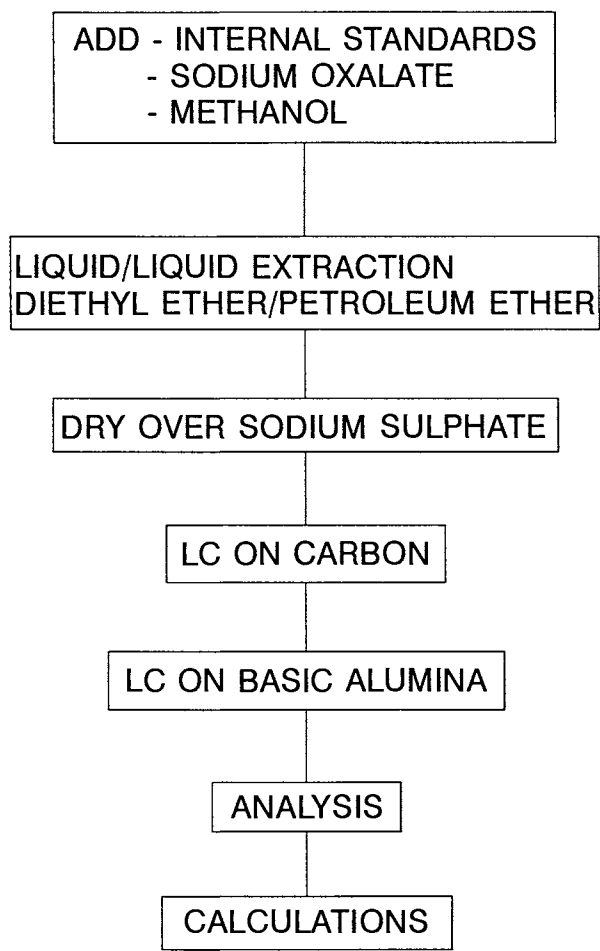


Figure 8: Clean-up scheme for cow's milk.

The most frequently used columns in dioxin analysis are DB5, Sil 5CB, HP 5 and Ultra 1 for non polar liquid phases and Silar 10C, CPSil 88, SP2331 and DB Dioxin for polar phases. Whatever column is found appropriate for the dioxin analysis, it must always be remembered that the column quality, which includes inertness as well as resolving power, is extremely important and must be rigorously checked by appropriate methods. Although such precaution might seem evident when dealing with columns in use, it is equally important to apply these criteria when accepting recently ordered columns. This is especially true when dealing with polar columns, for which it is still extremely difficult for the manufacturers to guarantee a constant resolution power. As an example, the performance offered by good quality polar columns towards dioxin determinations in fly ashes are listed in Table 8.

Table 8 Performance offered by different polar capillary columns for fly ash analysis.

(+) = adequately separated; (-) = not adequately separated; (*) = critical isomer suitable for control of column quality [115].

Isomer	SP 2331 CP Sil 88	DB Dioxin
Dioxins		
2,3,7,8	(+) (*)	(+) (*)
1,2,3,7,8	(+)	(-)
1,2,3,4,7,8	(+)	(+)
1,2,3,6,7,8	(+)	(+)
1,2,3,7,8,9	(+)	(+)
Dibenzofurans		
2,3,7,8	(+) (*)	(+) (*)
1,2,3,7,8	(-)	(+)
2,3,4,7,8	(+)	(-)
1,2,3,4,7,8	(-)	(+) (*)
1,2,3,6,7,8	(+)	(+)
1,2,3,7,8,9	(-)	(+)
2,3,4,6,7,8	(+)	(+)

24.1.3.2 Mass spectrometry

The selection of mass spectrometry as a detection method for the analysis of dioxins is based upon three specific characteristics of the mass spectra of these compounds, in combination with one more general advantage typical for mass spectrometric detection:

- the electron impact spectra show rather intense molecular ions at reasonably specific masses. So detection can be both sensitive and selective at the same time;
- the presence of four to eight chlorine atoms, for which there are two naturally occurring isotopes, results in typical ion clusters in the molecular ion region. The relative abundances of the ions in these clusters have to obey the general laws of probability and can therefore be calculated and checked;
- fragmentation, although weak, does occur in a very specific way, predominantly by the loss of COCl-fragments, and to a lesser extent of COCl-Cl₂-fragments, from the molecular ions. The resulting fragment ions are extremely selective and can be used for the confirmation of the dioxin identity;
- an additional, more general advantage of mass spectrometry is that it allows the simultaneous and selective detection of both native and isotopically labelled compounds. ³⁷Cl- or ¹³C-labelled dioxins and dibenzofurans are an ideal type of internal standards because they exhibit the same chemical properties as the native compounds and are absent in nature, two prime conditions to be fulfilled by any internal standard. Due to the rather complex nature of dioxin analysis in general, the use of such isotopically labeled isomers as internal standards for the determination of the seventeen 2,3,7,8-chlorinated dioxins and dibenzofurans has proved extremely useful, if not absolutely necessary. As a consequence it has now become general practice to spike the samples to be analysed with known amounts of at least one ¹³C₁₂-labelled congener per isomeric group, thus resulting in a total of ten ¹³C-labelled internal standards (five labelled dioxins and five labelled dibenzofurans). ³⁷Cl-labelled compounds are used much less frequently for this purpose.

In practice, monitoring of the three most abundant ions in the molecular ion clusters of both native and ¹³C-labelled dioxins will be the method of choice for dioxin and dibenzofuran analysis. In this technique, generally referred to as Selected Ion Monitoring or SIM, selective sets of ions are monitored in specific time windows, which depend upon the type of capillary column used for the gas chromatographic separation and its operating conditions. The sets of ions monitored for the different isomeric groups and the isotope peak ratios to be matched are listed in Table 9.

In principle, the choice between low and high resolution mass spectrometric detection, as available from quadrupole and sector type instruments, respectively, depends upon two conditions.

First, there is the complexity of the final cleaned-up sample, which must be free from interfering compounds, *i.e.* compounds that cannot be separated from the dioxins to be determined by the gas chromatographic column and cannot be distinguished by the mass spectrometric detection. It will be obvious that this condition is fulfilled more readily when analysing on a high resolution sector instrument (normal operating resolution = 10,000) than on a low resolution quadrupole type spectrometer (unit mass resolution). As a consequence, high resolution detection often allows appreciable simplifications of the required clean-up procedures. Secondly, the technique selected for detection should exhibit enough sensitivity in order to allow a reliable quantitation of the dioxin levels present in the sample. Although modern low resolution instruments are already able to reach detection limits as low as 500 fg in routine operation, the available high resolution instrumentation is still a factor of 25 to 50 more sensitive and normally allows limits of detection in the range of 10 to 20 fg.

Consequently, high resolution gas chromatography in combination with high resolution mass spectrometric detection is used routinely for dioxin and dibenzofuran analysis. In those situations where detection sensitivity does not pose any problem, low resolution mass spectrometric detection can be applied for the analysis of either relatively simple matrices, or more complex but well known and constant matrices for which adequate and proven clean-up procedures can be applied.

Table 9 Nominal masses normally traced and intensity ratios to be met in dioxin and dibenzofuran analysis.

Isomeric group	SIM masses	Intensity ratio's
T4CDD	320 / 322 / 324	76 / 100 / 50
¹³ C12-T4CDD	332 / 334 / 336	76 / 100 / 50
P5CDD	354 / 356 / 358	61 / 100 / 66
¹³ C12-P5CDD	366 / 368 / 370	61 / 100 / 66
H6CDD	388 / 390 / 392	51 / 100 / 82
¹³ C12-H6CDD	400 / 402 / 404	51 / 100 / 82
H7CDD	424 / 426 / 428	100 / 99 / 54
¹³ C12-H7CDD	436 / 438 / 440	100 / 99 / 54
OCDD	458 / 460 / 462	87 / 100 / 66
¹³ C12-OCDD	470 / 472 / 474	87 / 100 / 66
T4CDF	304 / 306 / 308	76 / 100 / 50
¹³ C12-T4CDF	316 / 318 / 320	76 / 100 / 50
P5CDF	338 / 340 / 342	61 / 100 / 66
¹³ C12-P5CDF	350 / 352 / 354	61 / 100 / 66
H6CDF	372 / 374 / 376	51 / 100 / 82
¹³ C12-H6CDF	384 / 386 / 388	51 / 100 / 82
H7CDF	408 / 410 / 412	100 / 99 / 54
¹³ C12-H7CDF	420 / 422 / 424	100 / 99 / 54
OCDF	442 / 444 / 446	87 / 100 / 66
¹³ C12-OCDF	454 / 456 / 458	87 / 100 / 66

24.1.3.3 Data evaluation

Calibrations and concentration calculations are performed according to the procedures normally used in the case of internal standardisation. First, isomer specific relative sensitivity factors (RSF's) are calculated from the analysis of a calibrant containing each of the native compounds to be determined in combination with the appropriate internal standards.

$$RSF_x = \frac{\sum AREA_{IS} (2 \text{ most abundant isotope peaks})}{\sum AREA_x (2 \text{ most abundant isotope peaks})} \cdot \frac{amount_x}{amount_{IS}}$$

Subsequently, the amount of each individual 2,3,7,8-chlorinated dioxin and dibenzofuran present in the original sample is calculated according to the following general formula:

$$Amount_{x,sample} = \frac{\sum AREA_x (2 \text{ most abundant isotope peaks})}{\sum AREA_{IS} (2 \text{ most abundant isotope peaks})} \cdot RSF_x \cdot Amount_{IS,added}$$

from which follows:

$$CONC_{x,sample} = \frac{Amount_{x,sample}}{Weight_{sample}}$$

24.2 Quality control and method validation

Quality control and method validation are necessary in order to check and prove the quality of the analytical results obtained in a laboratory on a day to day basis.

24.2.1 Internal quality control

24.2.1.1 Blanks for standard preparation equipment

All receptacles, syringes and other equipment used for standard preparation, should be checked first for possible contamination. It is good practice to prepare blank standards which are left overnight for equilibration. Taking into account the relatively simple dioxin compositions to be expected for glassware contaminated by former standard solutions, the screening of these blanks can be done quickly using a relatively short non-polar column in combination with electron capture detection. Problems with equipment showing unacceptable blanks can be greatly reduced by always using the same equipment for the same standard preparation. Materials that even then show unacceptable blanks, *i.e.* a contamination larger than 0.5 % of the calibrant concentration to be prepared, should be cleaned and rechecked until appropriate blank values are obtained.

24.2.1.2 Procedure blank

The number of simultaneously performed dioxin analyses differs slightly from one laboratory to another. It is however common practice to perform one arbitrarily chosen blank control for each set of extraction units that will be used simultaneously. The blank extract, to which all of the normal internal standards were added at the start, is further treated and analysed in exactly the same way as a real sample. Whenever a positive blank results, all of the equipment in the set must be cleaned and checked again until definitely negative blanks are obtained.

24.2.1.3 Spiking standards of $^{13}\text{C}_{12}$ -labelled compounds

The internal day to day quality control is based largely upon the use of the appropriate $^{13}\text{C}_{12}$ -labelled internal standards. Two types of internal standard solutions, both made up in n-nonane, must be available and must be prepared gravimetrically. The first, or so called quantification standard, is a mixture of five 2,3,7,8-chlorinated and $^{13}\text{C}_{12}$ -labelled dioxins and five 2,3,7,8-chlorinated and $^{13}\text{C}_{12}$ -labelled furans, one each time per isomeric group. Known amounts of this spiking standard are added to each sample or blank experiment before any treatment has taken place and the final results for these $^{13}\text{C}_{12}$ -labelled compounds are used for the quantification of the native dioxins present in the sample. The second is a single component solution exclusively containing $^{13}\text{C}_{12}$ -labelled 1,2,3,4-tetrachlorodioxin. It is added in known amounts to the final cleaned-up extracts just prior to GC-MS analysis and acts as a reference compound against which the recoveries of the other $^{13}\text{C}_{12}$ -labelled compounds can be calculated. As both of these solutions will be used over long periods of time, it is advisable to keep track of eventual solvent losses by accurate weighing in standard conditions. The $^{13}\text{C}_{12}$ -labelled compounds present in both spiking solutions must also be used as internal standards in the standard solutions used for calibration.

24.2.1.4 Calibration standards/detector linearity check

Five to six calibration standards, containing each of the native compounds to be determined in combination with the appropriate $^{13}\text{C}_{12}$ -labelled internal standards (*i.e.* those present in both spiking solutions), must be prepared gravimetrically. It is advisable to start this preparation from 5 to 10 $\mu\text{g.g}^{-1}$ stock solutions. The concentrations of the native compounds must be adjusted in such a way that they cover the entire range of quantification. The concentration of the internal standards must be in the same range but must be kept constant. As both the stock and the calibration solutions will be used over longer periods of time, it is advisable to keep track of eventual solvent losses by accurate weighing in standard conditions. The complete series of working standards will be prepared freshly every month just before they are used for the monthly linearity check of the mass spectrometric detector. One or two of these working standards are used for actual day to day calibration and quantification purposes. Their concentrations are checked every week against a certified primary standard and they are prepared freshly when needed.

24.2.1.5 *Quality control of chromatographic capillary columns*

All capillary columns used in dioxin analysis, but especially those with polar liquid phases, should be checked regularly for inertness and resolving power. It is a good procedure to pool cleaned-up extracts from previous analyses according to the types of samples from which they were prepared. These mixtures, each of which is qualitatively representative for a certain type of sample, can then be concentrated and/or diluted to different degrees so as to cover the range of concentrations regularly encountered for that type of analysis. Subsequently these so called qualitative reference samples can be used for checking the inertness and the chromatographic resolution of the capillary columns with respect to the samples to be analysed. Resolution should be checked on the basis of the separation obtained for a number of critical pairs. Inertness can be checked by monitoring, at an adequate concentration level, the peak shapes and the relative intensities of the different isomers present in the reference cocktail. Whenever there is an activity problem, different dioxin and dibenzofuran isomers tend to react differently thus resulting in a net change of relative peak intensities.

24.2.1.6 *Extraction efficiency*

Upon discussing the extraction efficiency, one must realize that it is never possible to be absolutely sure that an extraction method applied to a specific sample extracts all of the dioxins present. For liquid samples, for which it is reasonable to assume that the $^{13}\text{C}12$ -spikes added to the sample are in the same physical state as the native dioxins present, spike recovery values might still be a good indication of the minimal extraction efficiencies obtained. However, in the case of solid samples there might be very important differences in physical states between native compounds and spikes. In this case, spike recovery values just account for the losses occurring during extraction and clean-up and are no indication whatsoever for extraction efficiency. As a consequence, when setting up an extraction method for a specific type of sample, different techniques and methods must be compared and the one offering the highest relative efficiency will usually be selected. Subsequently, while practicing the developed methodology, the quality control related to extraction efficiency will be limited to a regular check of the effectiveness with which the chosen extraction methodology is applied. This is accomplished by re-extracting and re-analysing the same sample a second time.

$$E(\%) = 100 \frac{(X_1 - X_2)}{X_1}$$

$E(\%)$: the % of compound extracted in the first step relative to the total extractable amount if the method were applied with a 100 % efficiency.

X_1 and X_2 : amounts present in first and second extract.

It is good practice to perform this test routinely on each new series of samples of a different origin. No fixed indications can be given as to the limits between which this first step extraction efficiency is allowed to vary, as control limits largely depend upon the type of sample analysed as well as upon the dioxin levels to be determined. Problems regarding the percentage of compounds resulting from the first extraction step should lead to the modification of the standard or developed extraction procedure or eventually to the development and testing of a different procedure.

24.2.1.7 Recovery check

Spike recoveries are an indication of the quality with which the overall analytical procedure has been executed. In a first stage, based upon the data obtained from a calibration run, relative sensitivity factors are calculated for each spike relative to 1,2,3,4-¹³C12 tetrachlorodioxin as an internal standard:

$$RSF_{spike} = \frac{\sum AREA_{1,2,3,4} (2 \text{ most abundant isotope peaks})}{\sum AREA_{spike} (2 \text{ most abundant isotope peaks})} \cdot \frac{amount_{spike}}{amount_{1,2,3,4}}$$

Subsequently, based upon these RSF's and using the data obtained from the analysis of the real sample, the amounts of each spike present in the final cleaned-up sample can be calculated, using again 1,2,3,4-¹³C12tetrachlorodioxin as an internal standard:

$$Amount_{spike} = \frac{\sum AREA_{spike} (2 \text{ most abundant isotope peaks})}{\sum AREA_{1,2,3,4} (2 \text{ most abundant isotope peaks})} \cdot RSF_{spike} \cdot Amount_{1,2,3,4}$$

Finally, percent recoveries for each spike are obtained by comparing the amounts of spikes measured to the amounts added at the start of the analytical procedure:

$$\% \text{ RECOVERY}_{spike} = \frac{Amount_{spike \text{ measured}}}{Amount_{spike \text{ added}}} \cdot 100$$

It will be evident that the effort required for recovery determinations is minimal. Indeed, all of the required experimental data are available from calibration and sample analysis anyway. It is therefore advisable to include these recovery controls for each sample to be analysed. Again, no fixed indications can be given as to the limits between which % recoveries may be allowed to vary, as control limits largely depend upon the type of sample analysed as well as upon the dioxin levels to be determined. In general, the smaller the fluctuations in the recovery, the better the method used is under control.

24.2.1.8 Dioxin - dibenzofuran identification criteria

The unambiguous identification of the dioxins and dibenzofurans searched for is an important aspect of analytical quality control in dioxin analysis. In order to be identified as a specific 2,3,7,8-chlorinated dioxin or dibenzofuran, the following set of criteria must be fulfilled:

- signal to noise ratio ≥ 2.5
- the ions must occur in the correct retention time windows as determined from the analysis of a qualitative representative standard;
- correct relative retention time relative to the corresponding internal standard;
- correct chlorine isotope ratio's of the three most abundant isotope peaks;
- correct elemental compositions of the molecular ions as derived from the exact molecular masses;
- the occurrence of COCl loss by fragmentation.

24.2.1.9 Limits of detection (LD)

The limit of detection is defined as the minimum amount of a compound present in a sample, which still results in a signal to noise ratio of 2.5 upon final analysis. The weight of sample analysed, the sample preparation efficiency, the amount of chemical noise, the level of instrumental noise and instrumental sensitivity are all variables that may have a profound influence on the limits of detection for each sample and each isomer analysed.

The calibration run offers the data for the determination of the relative sensitivity factors based on peak heights.

$$RSF_x = \frac{Height_{IS}}{Height_x} \cdot \frac{amount_x}{amount_{IS}}$$

The limit of detection for a specific isomer, or in other words the concentration of an isomer in the original sample, corresponding with a signal of 2.5 times the baseline noise upon final analysis, is then calculated from the corresponding RSF and the data obtained from the analysis of the sample:

$$LD_x = \frac{Height_x \text{ at } 2.5 \times \text{baseline noise}}{Height_{IS}} \cdot RSF_x \cdot \frac{Amount_{IS}}{Amount_{sample}}$$

x: native isomer

Height: peak height of the most abundant molecular ion

IS: internal standard corresponding to x

LD: limit of detection of the overall analytical methodology in concentration units.

24.2.1.10 Precision and accuracy

Precision is defined as the degree to which an analytical method allows an experienced operator to reproduce the results obtained for a certain sample. The degree of precision required for a given analysis largely depends upon the context in which the results will be used or the type of problem to be solved. In practice, precision is determined by applying the appropriate statistics to the sets of results obtained from multiple analyses of one and the same sample. Practical day to day checking of precision is generally performed by analysing the same sample twice at regular intervals.

Accuracy is defined as the degree to which the result found for a specific sample by a specific method reflects reality. The only way in which accuracy can be routinely estimated is an internal quality control programme, consists of the analysis of certified reference materials of the same type as the samples at hand. Several of these reference materials are or will be available in the near future. The most important sources are the Community Bureau of Reference (BCR, CEC) and the National Institute of Standards and Technology (NIST, USA).

24.2.1.11 Internal quality control systems

Obviously, the organisation of a reliable quality control system for dioxin analysis does not necessarily require the inclusion at all times of all of the checks discussed in the previous pages. The number of controls required and the frequency of their execution will depend upon a number of parameters such as the type of samples to be analysed, the quality of the chemicals and materials used for extraction and clean-up, instrument stability, organisation of the analytical laboratory and operator experience. As a consequence, the setting-up of an internal quality control system should in the first place be a learning process from which even major improvements in laboratory organisation and analytical methodologies themselves might result. The finally developed validated control system should be simple but efficient and should keep a controlled flexibility for improvements at all times.

24.2.2 Method validation

Taking part in national and international inter-laboratory comparisons, thus confronting the shortcomings and qualities of ones own methodology with those of the methods applied on the same samples in other laboratories, is by far the best way to validate an analytical method. Interlaboratory comparisons have been and are still being organised by several organisations such as the WHO, the NIST or the BCR. Because the final goal of these exercises often lies in the certification of specific reference materials, they are conducted in an extremely critical way and therefore are the ultimate forum for method validation. The experiences gathered upon validation within a European network of the method developed in our own laboratory for the analysis of the dirty dozen dioxins and dibenzofurans in fly ashes from municipal waste incinerators were extremely positive. External quality control and final validation for that method were performed within the framework of a programme set-up and sponsored by the CEC and aiming at the preparation and certification of a fly ash reference material for dioxins and dibenzofurans. All exercises were organised according to the overall interlaboratory quality control scheme shown in Fig. 9.

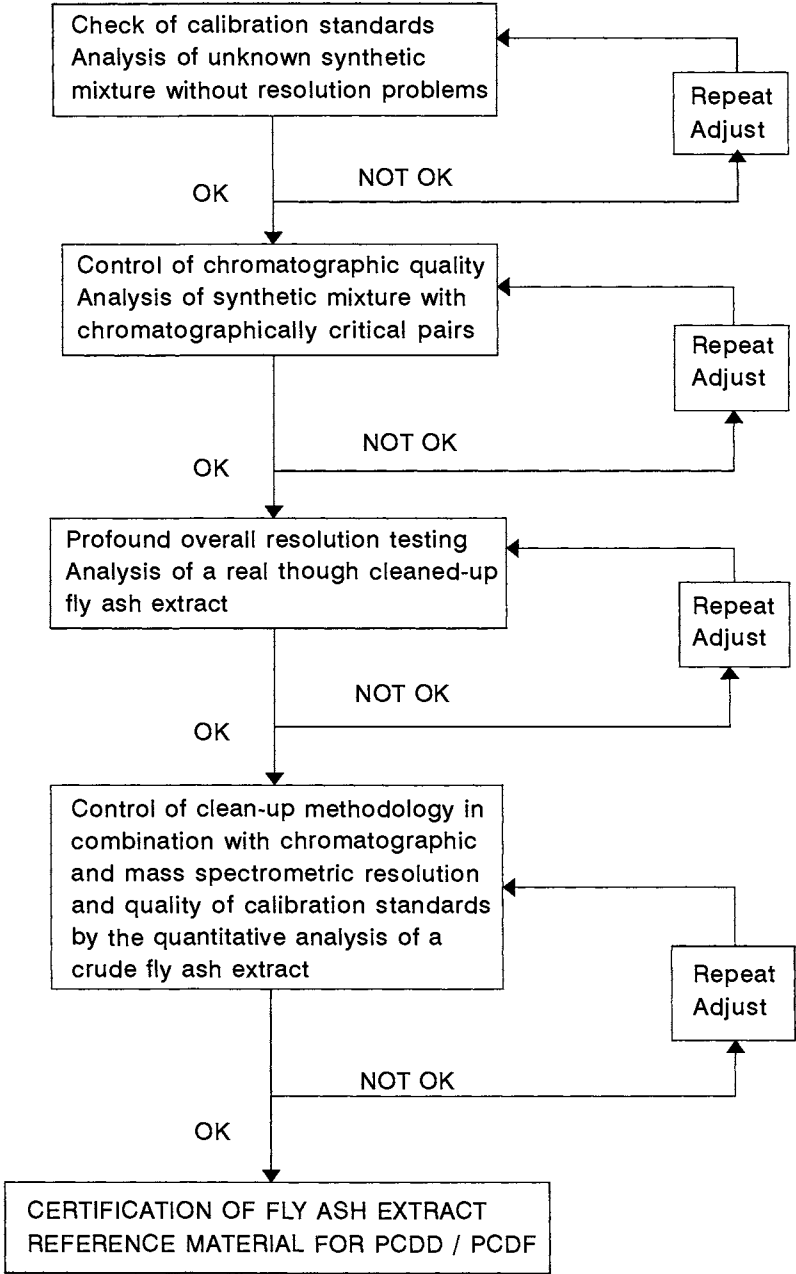


Figure 9: The overall interlaboratory quality control scheme followed during the BCR exercises.

In the final exercise as well as for actual certification of a fly ash extract, each participant was obliged to perform the analysis according to a fixed set of internal quality criteria, which were as follows:

- execution of five independent analyses;
- check of the method blank, that should be blank at the concentration levels of interest;
- check of detector linearity in the appropriate concentration range, for each isomer and using at least five different calibration solutions. A very rough indication of the upper limits of these concentration ranges was communicated in advance to each participant;
- determination of recovery figures for each isomer to be determined. When recoveries for different isomers of the same isomeric group were found to be constant within the uncertainty limits of the experimental measurements, no further action nor corrections were required. Whenever the differences in recovery between different isomers of the same isomeric group were greater than could be explained on the basis of the experimental uncertainty, four replicate recovery determinations had to be performed and the final analytical results had to be corrected on the basis of the average recovery figures;
- blank analysis, calibration and sample analysis had to be performed so as to bracket the samples using the following scheme:

For sample N=1 to 5

- blank control
- calibration
- analysis of sample N

Next sample N+1

- for each sample, the final calculation had to be based on the average calibration data obtained from the two such calibration runs.

This set of controls was mandatory but could be complemented with other additional tests as found appropriate by the respective laboratories.

Each of these exercises revealed a number of small problems, that, once discovered, allowed rather simple and easy solutions. Also experienced participants had the benefit of a number of general as well as more specific findings:

- the impact upon analytical quality of using calibration standards that are referenced against certified reference standards, which are prepared gravimetrically starting from crystalline materials of verified purity, was proven;
- although the clean-up required for a fly ash extract is linked directly to the use of low or high resolution mass spectrometric detection, there is, in both cases, a rather broad range of valid clean-up procedures. As a consequence, selection should be based on simplicity and speed;
- the chromatographic separation of the so called "dirty dozen" from other isomers and accompanying impurities requires the analysis of the cleaned-up fly ash extract to be performed on at least two capillary columns. With the available column technology, the combination of a DB Dioxin column and another polar column of the type of SP 2331 or CP Sil 88 is the best choice (cf. Table 8).

However, column quality in view of dioxin analysis is very critical and therefore, in order to get optimum performance, it should be checked rigorously and regularly using appropriate qualitative reference samples.

As a result of these efforts, the methods applied in eleven different European laboratories for the analysis of fly ashes were validated. The overall quality of these methods is illustrated in table 10, which lists the averages and coefficients of variation of the data produced by these eleven participants for the final certification analysis.

Table 10: Averages (Av) and coefficients of variation (CV) obtained in the certification of a fly ash extract.

Compound (*)	Av (ng.g ⁻¹)	CV(%)
2,3,7,8-T4CDD	4.8	8.3
1,2,3,7,8-P5CDD	24.8	6.5
1,2,3,4,7,8-H6CDD	66	9.1
1,2,3,6,7,8-H6CDD	145	3.5
1,2,3,7,8,9-H6CDD	79	5.1
2,3,7,8-T4CDF	16.2	6.8
1,2,3,7,8-P5CDF	40.7	6.9
2,3,4,7,8-P5CDF	71	7.0
1,2,3,6,7,8-H6CDF	165	11
1,2,3,7,8,9-H6CDF	15.2	11.2
2,3,4,6,7,8-H6CDF	299	9.7

(*) 1,2,3,4,7,8 H6CDF, when determined on normal polar columns, is not free from interferences. The number of data available from DB Dioxin columns was not sufficient to allow certification.

24.3 Validated methods

In order to fulfil the requirements imposed by good laboratory practices, validated methods and corresponding procedures for quality control and quality assurance must be written down in full detail in analytical protocols and included in the laboratory quality assurance manual. These measures are then to be followed rigorously in the day to day practice. The following is a detailed description of such a final fully detailed procedure for the analysis of the twelve most toxic 2,3,7,8-chlorinated dioxins and dibenzofurans (the dirty dozen) in fly ashes from municipal waste incinerators.

24.3.1 Analytical procedure for the quantitative determination of 2,3,7,8-chlorinated tetra-, penta- and hexachloro dioxins and dibenzofurans in municipal waste incinerator fly ashes

Dioxins and dibenzofurans are toxic and should therefore be handled only according to the general guidelines applicable for radioactive and infectious materials. As an elaborate description of the general requirements regarding infrastructure and overall health and safety protocols is beyond the scope of this book, the analytical procedure described here will only include specific safety precautions relevant for fly ash analysis. Moreover, the references regarding the brands of instrumentation and chemicals used, although normally required in a fully detailed description of an analytical procedure written down in the laboratory protocol, will be omitted. In practice several brands and quality types of products, when checked properly, might be proven to be suitable for the analysis at hand. Finally, it is important to indicate that the procedure described hereafter assumes that samples are analysed in a series of four.

24.3.1.1 Cleaning of glassware

All glassware must be cleaned by applying the following sequence:

- (a) soak in hot, freshly prepared sulphochromic acid for 24 h. Sulphochromic acid preparations and manipulations must be performed in a fume hood, wearing protective clothes, gloves and glasses;
- (b) flush with tap water followed by bidistilled water;
- (c) rinse three times with acetone;
- (d) rinse three times with n-hexane;
- (e) dry non Soxhlet glassware overnight in a forced air oven at 350 °C.
- (f) set-up the Soxhlet units and extract for two hours with toluene.

24.3.1.2 *Materials and reagents*

Glassware:

- Soxhlet extraction units with 50 ml extraction capacity and 100 ml round bottom receivers;
- glass columns, 10 mm internal diameter and 30 cm long, provided with a glass frit at the bottom end;
- Pasteur pipettes, Erlenmeyers with a volume of 50 ml and sample vials with glass insert tips. The inserts can hold a maximum volume of 1 ml, while the lower tip volume is approximately 50 μ l.

Balance: analytical balance, properly checked and calibrated against a weight with certified mass.

Water: water free from organic impurities. It can be purchased or prepared in the laboratory using one of the many commercially available units.

Glasswool: glasswool is pre-cleaned by washing three times with acetone, followed by a triple washing in n-hexane and baking overnight at 350 °C in a forced air oven.

Toluene: distilled in glass.

Benzene: analytical quality.

n-Hexane: for residue analysis.

Dichloromethane: for residue analysis.

Acetone: for residue analysis.

Soxhlet thimbles: pre-cleaned by Soxhlet extraction in toluene for 2 h.

Filter paper: 185 mm diameter.

Nitrogen: nitrogen gas purified over activated carbon.

Na₂SO₄: anhydrous, prepared by heating in relatively thin layers at 140 °C for at least 8 h, and stored at room temperature in a desiccator.

Alumina: basic alumina, Super I activity, is used as delivered. The activity is checked using a test mixture containing 60 mg of 4-N,N'-dimethylaminoazobenzol, 60 mg of ceresred BB, 60 mg of indophenol and 40 mg of guaiazulene in 100 ml of CCl₄. 5 ml of this mixture are loaded on top of a 1 cm internal diameter column packed with 10 ml of alumina. Activity grade Super I is guaranteed when elution of the column with 15 ml of CCl₄ does not result in any migration of any of the compounds present.

Standards and spiking solutions: standards and spiking solutions all have a unique code. The weight of the empty receptacles, which will afterwards be used for the storage of the standard solutions, must be known. Subsequently, the flasks containing the standard solutions are weighed accurately, in standard conditions, each time before and after use. The weighing data are noted in a logbook and are used in order to keep track of unacceptable solvent losses during storage. Correction, by the addition of pure solvent, is performed when the weighing data indicate a relative change in solvent weight of more than 0.5 %.

¹³C-labelled internal standards: the spiking solution is a mixture, made up in n-nonane, of the twelve ¹³C-labelled internal standards corresponding to the twelve native compounds to be determined. The concentration of each ¹³C-labelled congener, which must be known exactly, must be close to 50 pg/μl.

Recovery standard: consists of ¹³C-labelled 1,2,3,4-T4CDD dissolved in n-nonane at an exactly known concentration close to 35 pg/μl.

Calibration standards: a series of five standard solutions, made up in n-nonane and containing variable concentrations of each of the twelve native compounds to be determined and fixed concentrations of each of their ¹³C-labelled analogues as well as of the ¹³C-labelled recovery standard. The concentration range covered by the native compounds must be between 1 and 300 pg/μl. The fixed concentrations of the ¹³C-labelled compounds (internal standards and recovery standard) must be close to 5 pg/μl.

DB-Dioxin capillary column: polar column, 60 m long, 0.25 mm internal diameter and with a coating film thickness of 25 μm. Before use, each column must be tested for its quality using a representative qualitative fly ash extract test mixture.

CP Sil 88 capillary column: polar column, 50 m long, 0.25 mm internal diameter and with a coating film thickness of 25 μm. Before use, each column must be tested for its quality using a representative qualitative fly ash extract test mixture.

24.3.1.3 *Method blank*

- Load one arbitrarily chosen Soxhlet unit with a pre-cleaned extraction thimble.
- Spike the thimble with 25 μl of the solution containing the twelve ¹³C-labelled internal standards.
- Close the thimble with a plug of pre-cleaned glasswool.
- Extract for 40 h with 80 ml of toluene.
- Proceed in exactly the same way as described hereafter for the analysis of real samples.

The method blank should be blank when analysed at the levels of sensitivity required for the analysis of the unknown samples. If not, then the cleaning of the entire set of equipment must be repeated.

24.3.1.4 Sample analysis

a) Pretreatment, extraction and clean-up

- Weigh 1 g of fly ash in an Erlenmeyer and add 20 ml of 4 % hydrochloric acid. The suspension must be homogenized for 2 h using a magnetic stirrer.
- The suspension is filtered over paper, taking care that the material transfer occurs quantitatively.
- Wash the solid material with ultra-pure water until a neutral filtrate is obtained.
- Shield the acid treated fly ash and allow it to dry at room temperature.
- Transfer the sample quantitatively, filter paper included, into the pre-cleaned Soxhlet thimble and mount it into the Soxhlet extraction apparatus.
- Add 25 μ l of the internal standard solution and top the thimble with a plug of pre-cleaned glasswool.
- Extract the sample for 40 h with 80 ml of toluene.
- Concentrate the extract, by using a gentle stream of ultra clean, dry nitrogen, to a final volume of approximately 2 ml.
- Weigh 2.5 g of alumina, super I activity, checked as described before, and feed it into a glass column, 10 mm internal diameter and 30 cm long, under gentle vibration.
- Weigh 2 g of anhydrous Na_2SO_4 and apply it to the top of the alumina column.
- Using small portions of benzene as a solvent for repetitive washing, quantitatively transfer the concentrated extract to the top of the column and elute the column consecutively with a total volume of 15 ml of benzene (fraction I), 20 ml of n-hexane/dichloromethane 98/2 (V/V) (fraction II) and 30 ml of n-hexane/dichloromethane 50/50 (V/V) (fraction III), taking care to collect the three fractions separately.
- Discard fractions I and II.
- Concentrate fraction III under a gentle stream of dry, ultra clean nitrogen to a final volume of approximately 1 ml.
- Using dichloromethane as a washing solvent, quantitatively transfer the concentrate to a glass insert tip placed in a sample vial and concentrate the cleaned-up extract to a final volume of approximately 20 μ l.
- Add 30 μ l of ^{13}C 12-1,2,3,4-T4CDD recovery standard solution and mix thoroughly by sonication.

b) GC - MS analysis

- Check the gas chromatographic and mass spectrometric operating conditions which should be as indicated in table 11.

Table 11: Gas chromatographic and mass spectrometric operating conditions.

GC-column		
Type	DB-Dioxin	CP Sil 88
Length	60 m	50 m
Internal diameter	0.25 mm	0.25 mm
Film thickness	0.25 μm	0.25 μm
Temp. program	stay at 160 °C for 1 min; to 240 °C at 20 °C min ⁻¹ ; stay 35 min; to 270 °C at 15 °C min ⁻¹ ; stay 28 min.	stay at 110 °C for 1 min; to 170 °C at 30 °C min ⁻¹ ; stay 35 min; to 230 °C at 3 °C min ⁻¹ ; stay 2 min;
Injection		
Type	splitless	splitless
Split time	1 min	1 min
Injection volume	1 μl	1 μl
Temperature	270 °C	270 °C
Carrier gas	He	He
Inlet pressure	200 kPa	180 kPa
GC-MS		
Type	high resolution	high resolution
Interface	direct	direct
Interface temp.	270 °C	270 °C
Source type	EI+	EI+
Source temp.	250 °C	250 °C
Filament current	3800 mA	3800 mA
Electron energy	34 eV	34 eV
Operating mode	SIM	SIM
Mass calibration	perfluorokerosene	perfluorokerosene

- Fine tuning of the mass spectrometer to maximum sensitivity at 10.000 resolution (10 % valley definition) must be carried out on a daily basis, using perfluorokerosene (PFK) as a reference compound.
- Check the logbook for the linearity control sequence of the GC - MS response. If necessary, perform a linearity check first.
- Perform the analyses on each series of 4 samples (any one of which could be a reference material or an additional control blank) according to the following scheme (the sequence in which the capillary columns are used may be reversed, *i.e.* the column that is mounted in the instrument at the start of a new series of samples will be used first):

Day 1: analysis on a DB-Dioxin column:

- calibration solution;
- syringe blank control (check immediately (*));
- 4 consecutive samples;
- calibration solution.

Day 2: analysis on a CP Sil 88 column:

- calibration solution;
- syringe blank control (check immediately (*));
- 4 consecutive samples;
- calibration solution.

(*) Obviously, the syringe blank controls executed in each sequence before starting the analysis of the real samples require an immediate check and eventual action. Analysis of the unknown samples should not be started as long as the blank control is not really blank at the level of sensitivity required for the unknown samples.

c) Data evaluation

All of the analytical parameters calculated have to be introduced into the corresponding control charts of the quality control system and have to be checked for compliance with previously set quality control limits. When the respective quality control conditions are fulfilled, the calculations can proceed. If problems occur, such as too large a fluctuation in calibration data or unacceptable results for the additional control blank or reference material included as an unknown in the set of samples, then all of the data of the corresponding run have to be discarded and proper remediating actions should be taken.

- Calculate, from first and second calibration runs, the instrumental lower limits of detection at the start and at the end of the day.

$$LD_{ms,x} = \frac{Height_x \text{ (at } 2.5 \times \text{ baseline noise)}}{Height_{x,S}} \cdot Amount_{x,S}$$

x: isomer

Height_x (at 2.5 x baseline noise): peak height of x corresponding to 2.5 x the baseline noise.

Height_{x,S}: peak height of the most abundant molecular ion of x upon calibration.

Amount_{x,S}: absolute amount of x injected upon calibration (in pg).

LD_{ms,x}: instrumental limit of detection for x in pg.

- Feed the results to the corresponding control charts and check for their compliance.
- Calculate the recoveries for the ¹³C-labelled internal standards relative to the ¹³C-labelled 1,2,3,4-T4CDD added after the clean-up.

First calculate the required RSF's from the data of the calibration runs:

$$RSF_{spike} = \frac{\sum AREA_{1,2,3,4} \text{ (2 most abundant isotope peaks)}}{\sum AREA_{spike} \text{ (2 most abundant isotope peaks)}} \cdot \frac{amount_{spike}}{amount_{1,2,3,4}}$$

Subsequently calculate the individual recoveries from the data obtained for the unknown sample.

$$Amount_{spike} = \frac{\sum AREA_{spike} \text{ (2 most abundant isotope peaks)}}{\sum AREA_{1,2,3,4} \text{ (2 most abundant isotope peaks)}} \cdot RSF_{spike} \cdot Amount_{1,2,3,4}$$

% RECOVERY_{spike} = $\frac{Amount_{spike\ measured}}{Amount_{spike\ added}} \cdot 100$

The recoveries, which must also be fed to the appropriate quality control charts, should fulfil the requirements listed in table 12.

Table 12: Recovery requirements for the analysis of an average fly ash.

Compound	% Recovery limits
T4CDD	> 60
P5CDD	> 50
H6CDD	> 40
T4CDF	> 60
P5CDF	> 50
H6CDF	> 40

- Identify the dioxins and dibenzofurans present in calibration solutions, blanks and unknown samples on the basis of the following identification criteria:
 - . signal to noise ratio ≥ 2.5;
 - . the ions must occur in the correct retention time windows;
 - . correct retention time of the isomer relative to the corresponding internal standard;
 - . correct chlorine isotope ratio's of the three most abundant isotope peaks;Calculate for each isomer the two RSF's resulting from the two daily calibrations:

$$RSF_x = \frac{\sum AREA_{IS} \text{ (2 most abundant isotope peaks)}}{\sum AREA_x \text{ (2 most abundant isotope peaks)}} \cdot \frac{amount_x}{amount_{IS}}$$

- Check the RSF data against the quality control requirements and , if valid, calculate the average RSF for each isomer, which will be used for further calculations.

- Calculate the concentrations in the unknown samples. The calculations must be based upon the data generated by the DB-Dioxin column for all congeners except for 1,2,3,7,8-P5CDD and 2,3,4,7,8-P5CDF, for which the calculation is based upon the data obtained from the CP Sil 88 column.

$$\text{Amount}_{x,\text{sample}} = \frac{\sum \text{AREA}_x (2 \text{ most abundant isotope peaks})}{\sum \text{AREA}_{IS} (2 \text{ most abundant isotope peaks})} \cdot \text{RSF}_x \cdot \text{Amount}_{IS,\text{added}}$$

from which it follows:

$$\text{CONC}_{x,\text{sample}} = \frac{\text{Amount}_{x,\text{sample}}}{\text{Weight}_{\text{sample}}}$$

- Finally the data will be checked by the quality control manager for the results related to hidden blanks, duplicate samples and hidden reference samples. All QA data are fed to the quality control system and checked for compliance. Again, blanks should be blank at the sensitivity levels of interest. The results for the reference samples should be within the 95 % confidence interval of the certified values. For an average municipal waste incinerator fly ash, the coefficient of variation calculated on the basis of duplicate samples should always be less than 25 %.

24.4 Conclusions

The saying that: "accurately measuring things is equal to knowing things" most certainly holds for the evaluation of the worldwide dioxin problem. However, measuring accurately is not at all that easy. It requires a lot of skill and personnel motivation for quality in addition to proven analytical methodologies and appropriate laboratory quality control systems. Two aspects, which have a profound effect on the overall analytical quality, are the availability of accurately certified reference standards for native as well as ¹³C-labelled dioxins and dibenzofurans, and the opportunity to use accurately certified reference materials for initial method development as well as for day to day quality control purposes. Although certified standards, in a crystalline form as well as in solution, are readily available on the market, the same does not hold in the field of certified reference materials. So, although the preparation and subsequent certification of reference materials requires an important and combined effort from several laboratories, and consequently can only be performed at an appreciable cost, there is a substantial need for these materials, without which dioxin analysis will never be able to reach an acceptable level of quality as required for scientific based decisions.

The availability of analytical methods as such may be solved. Indeed, methods have been developed for the analysis of dioxins in various types of matrices. The main disadvantage of these methods is often their extreme complexity. As a consequence, they are labour intensive, time consuming, costly and carry considerable analytical risks. So, the major progress in the field of dioxin analysis may be expected in the development of new and faster methods for sample extraction and sample preparation. But it will also be necessary to validate these methods against the proper certified reference materials.

Finally, if sample preparation can develop such that it allows the elimination of all mass spectrometric interferences, a further increase in the detection sensitivity of the cheaper quadrupole type mass spectrometric detectors could be a major breakthrough. Such an evolution would not only lead to an important reduction of the cost of dioxin analysis but it would also make the field of dioxin analysis more generally accessible.

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